(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 613 945 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
03.05.2000 Bulletin 2000/18

(21) Application number: **94301367.2**

(22) Date of filing: **25.02.1994**

(51) Int. Cl.[7]: **C12N 15/12**, C12P 21/08, C12Q 1/68, G01N 33/574, A61K 48/00, C07K 14/82

(54) Tumor suppressor gene merlin and uses thereof

Tumorsuppressor-Gen Merlin und dessen Verwendungen

Merlin, gène suppresseur de tumeurs, et ses utilisations

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **25.02.1993 US 22034**
04.03.1993 US 26063
19.08.1993 US 108808
22.12.1993 US 171718

(43) Date of publication of application:
07.09.1994 Bulletin 1994/36

(83) **Declaration under Rule 28(4) EPC (expert solution)**

(73) Proprietor:
THE GENERAL HOSPITAL CORPORATION
Boston, MA 02114 (US)

(72) Inventors:
- **Trofatter, James A.**
  Charlestown, Massachusetts 02129 (US)
- **MacCollin, Mia M.**
  Medford, Massachusetts 02155 (US)
- **Gusella, James F.**
  Framingham, Massachusetts 01701 (US)

(74) Representative:
Bassil, Nicholas Charles et al
Kilburn & Strode
20 Red Lion Street
London WC1R 4PJ (GB)

(56) References cited:
- **CELL 72 (5). 1993. 791-800. CODEN: CELLB5 ISSN: 0092-8674, TROFATTER J A ET AL 'A NOVEL MOESIN ERZIN RADIXIN-LIKE GENE IS A CANDIDATE FOR THE NEUROFIBROMATOSIS 2 TUMOR SUPPRESSOR.'**
- **JAMA (JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION) 270 (19). 1993. 2316-2320. ISSN: 0098-7484, MACCOLLIN M ET AL 'DNA diagnosis of neurofibromatosis 2: Altered coding sequence of the merlin tumor suppressor in an extended pedigree.'**
- **NATURE GENETICS 6 (2). 1994. 185-192. ISSN: 1061-4036, BIANCHI A B ET AL 'Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumor types.'**
- **NATURE, vol. 363, 10 June 1993 pages 515-521, G.A. ROULEAU ET AL 'Alteration in a new gene encoding a putative membrance-organizing protein causes neuro-fibromatosis type 2'**
- **AM J HUM GENET 46 (2). 1990. 323-328. CODEN: AJHGAG ISSN: 0002-9297, ROULEAU G A ET AL 'FLANKING MARKERS BRACKET THE NEUROFIBROMATOSIS TYPE 2 NF2 GENE ON CHROMOSOME 22.'**
- **KEYSTONE SYMPOSIUM ON MELANOMA AND BIOLOGY OF THE NEURAL CREST, TAOS, NEW MEXICO, USA, FEBRUARY 1-8, 1992. J CELL BIOCHEM SUPPL 0 (16 PART B). 1992. 306. CODEN: JCBSD7, SEIZINGER B R ET AL 'TUMOR SUPPRESSOR GENES AND NEURAL CREST-DERIVED NEOPLASMS TOWARD THE ISOLATION OF THE GENE CAUSING NEUROFIBROMATOSIS TYPE 2 NF2.'**

(Cont. next page)

EP 0 613 945 B1

- PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET 49 (4 SUPPL.). 1991. 384. CODEN: AJHGAG ISSN: 0002-9297, MENON A G ET AL 'PHYSICAL MAPPING OF THE NEUROFIBROMATOSIS TYPE 2 NF-2 LOCUS ON CHROMOSOME 22.'
- TENTH INTERNATIONAL WORKSHOP ON HUMAN GENE MAPPING, NEW HAVEN, CONNECTICUT, USA, JUNE 11-17, 1989. CYTOGENET CELL GENET 51 (1-4). 1989. 1070. CODEN: CGCGBR ISSN: 0301-0171, ROULEAU G A ET AL 'PRECISE MAPPING OF THE NEUROFIBROMATOSIS TYPE 2 LOCUS ON CHROMOSOME 22.'

Description

Field of the Invention

[0001] The invention is in the field of genetic disease diagnosis, tumor detection and treatment, and genetic therapy. Specifically, the invention is directed to the *merlin* gene, *merlin* protein, and the use of the gene and/or protein for (1) detecting a predisposition to develop tumors especially neurofibromatosis-2, (2) diagnosing certain tumors, especially neurofibromatosis-2, (3) treating tumors, especially neurofibromatosis-2, (4) monitoring the course of tumor treatment, especially neurofibromatosis-2 treatment, and (5) gene replacement in affected non-tumor tissues or cells.

Background of the Invention

[0002] Neurofibromatosis (NF) describes two major human genetic disorders that display autosomal dominant inheritance, involve tumors of the nervous system, and which are distinct clinical entities (Mulvihill, J.J. *et al., Ann. Intern. Med. 113*:39-52 (1990)). NF1, or von Recklinghausen NF, is more common (incidence of 1/4,000) and is characterized by the highly variable expression of an array of features that include neurofibromas, cafe-au-lait macules, Lisch nodules of the iris, and a predisposition to certain malignant tumors (Riccardi, V.M. *et al., N. Engl. J. Med. 305*:1617-1627 (1981); Riccardi, V.M. *et al.*, "Neurofibromatosis: Phenotype, Natural History, and Pathogenesis," Johns Hopkins Univ. Press, Baltimore, MD (1986)). It is caused by defects in a gene on chromosome 17 that has recently been isolated and characterized (Viskochil, D. *et al., Cell 62*:187-192 (1990); Cawthon, R.M. *et al., Cell 62*:193-201 (1990); Wallace, M.R. *et al., Science 249*:183-186 (1990)). The *NF1* gene product, neurofibromin, is a large protein with a GAP-related domain and is presumably involved in modulating a signal transduction pathway whose disruption can lead to tumor formation (Ballester, R. *et al., Cell 63*:851-859 (1990); Buchberg, A.M. *et al., Nature 347*:291-294 (1990); Xu, G. *et al., Cell 62*:599-608 (1990); DeClue, J.E. *et al., Cell 69*:265-273 (1992); Basu, T.N. *et al., Nature 356*:713-715 (1992)).

[0003] In contrast, neurofibromatosis-2 (NF2), which occurs in about 1/40,000 livebirths (Evans, D.G.R. *et al., J. Med. Genet. 29*:841-846 (1992)), is characterized by bilateral schwannomas that develop on the vestibular branch of the 8th cranial nerve. Pressure from these tumors often causes hearing loss and vestibular symptoms in the second and third decade. Other tumors of the brain, especially meningiomas and schwannomas of other cranial nerves and spinal nerve roots (Martuza, R.L. *et al., N. Engl. J. Med. 318*:684-688 (1988)), and posterior capsular lens opacities (Kaiser-Kupfer, M.I. *et al., Arch. Ophthalmol. 107*:541-544 (1989) are commonly present in the young affected adult.

[0004] The *NF2* gene is highly penetrant. Ninety-five percent of persons with the genotype develop bilateral vestibular schwannomas. NF2 is often more severe than NF1. Teenage or early adulthood onset of multiple slow growing tumors that can gradually cause deafness, balance disorder, paralysis or increasing neurologic problems necessitating repeated surgical procedures, characterizes NF2.

[0005] NF2 has been shown to be genetically distinct from NF1 by linkage studies that assigned the *NF2* gene to chromosome 22 (Rouleau, G.A. *et al., Nature 329*:246-248 (1987); Wertelecki, W. *et al., N. Engl. J. Med. 319*:278-283 (1988); Rouleau, G.A. *et al., Am. J. Hum. Genet. 46*:323-328 (1990); Narod, S.A. *et al., Am. J. Hum. Genet. 51*:486-496 (1992)). The tumor types that occur in NF2 can be seen in the general population as solitary, sporadic tumors. Since frequent loss of alleles on chromosome 22 from both sporadic vestibular schwannomas and meningiomas, and from their counterparts in NF2 had been noted previously, the localization of the inherited defect to the same chromosome region suggested that the *NF2* locus encodes a recessive tumor suppressor gene (Knudson, A.G. *et al., Proc. Natl. Acad. Sci. U.S.A. 68*:820-823 (1971)) whose inactivation leads to tumor formation (Seizinger, B.R. *et al., Nature 322*:644-647 (1986); Seizinger, B.R. *et al., Science 236*:317-319 (1987); Seizinger, B.R. *et al., Proc. Natl. Acad. Sci. U.S.A. 84*:5419-5423 (1987).

[0006] A number of studies of sporadic tumors and tumors from NF2 patients have provided support for this hypothesis (Couturier, J. *et al., Cancer Genet. Cytogenet. 45*:55-62 (1990); Rouleau, G.A. *et al., Am. J. Hum. Genet. 46*:323-328 (1990); Fiedler, W. *et al., Genomics 10*:786-791 (1991); Fontaine, B. *et al., Ann. Neurol. 29*:183-196 (1991); Fontaine, B. *et al., Genomics 10*:280-283 (1991); Bijlsma, E.K. *et al., Genes Chromosom. Cancer 5*:201-205 (1992); Wolff, R.K. *et al., Am. J. Hum. Genet. 51*:478-485 (1992)). The combined use of family studies and tumor deletion mapping has progressively narrowed the location of *NF2* within the q12 band of chromosome 22, and defined a candidate region in which to search for the NF2 genetic defect (Rouleau, G.A. *et al., Am. J. Hum. Genet. 46*:323-328 (1990); Wolff, R.K. *et al., Am. J. Hum. Genet. 51*:478-485 (1992)).

Brief Description of the Drawings

[0007]

Figure 1. Pulsed-field gel analysis of lymphoblast DNA from NF2 patients. DNA in agarose blocks was digested with NotI, subjected to electrophoresis, blotted, and hybridized to a radiolabeled 4 kbp *NEFH* probe. Lane 1, GUS6274 (affected NF2 unrelated to lanes 3 and 4); lane 2, GUS7870 (normal human); lane 3, GUS5068 (affected daughter); lane 4, GUS5069 (affected mother).

Figure 2. Physical map of chromosome 22 region surrounding the NF2 deletions. NotI sites were determined by pulsed-field gel analysis and confirmed in cosmids. Cosmids were named according to their standardized library coordinates. The enlarged region shows details of the *merlin* gene vicinity with deleted regions denoted by underlying brackets. Probes used in deletion analysis: A, 14 kbp HindIII fragment from cosmid 96C10; B, 8.5 kbp HindIII fragment from 28H6 that spans the T3 end of 96C10; C, 8 kbp HindIII end-fragment from the T7 end of 96C10; 96T3 (primer pair:

5'CAGATTGTTCATTCCAAGTGG3' [SEQ ID No 1] and
5'ACCCTGAGGAATCCACTACC3' [SEQ ID No: 2],

product size- 124 bp) and 96T7

(5'TGCACACACATCCTTTTCAC3' [SEQ ID No: 3], and
5'GAGAGAGACTGCTGTCTCAAAAA3' [SEQ ID No: 4],

product size-92 bp) are sequence-tagged site assay (STS)-derived from the T3 and T7 ends of 96C10, respectively. X-XhoI recognition site. Arrows indicate the orientation of transcription and approximate genomic coverage of cDNA JJR-1.

Figure 3A to 3F. Complete nucleotide and predicted amino acid sequence of *merlin* JJR-1 cDNA clone [SEQ ID No: 15 and 16, respectively] . Common moesin-ezrin-radixin domain spans amino acid residues 1-358. Arrow indicates where poly-A addition has occurred in two independent clones (JJR-6 and JJR-9).

Figure 4. Northern blot analysis of RNA derived from human tumor cell lines. RNA was size-fractionated by electrophoresis in formaldehyde-agarose, blotted and hybridized as described in Experimental Procedures. The blot was hybridized with a radiolabeled probe corresponding to bases 1253-1942 of the JJR-1 cDNA. Lane 1, SK-N-NB neuroblastoma; lane 2, T98G glioblastoma; lane 3, WERI retinoblastoma; lane 4, MCF-7 breast carcinoma; lane 5, HOS osteosarcoma; lane 6, HEPG2, liver carcinoma; lane 7, CATA4 kidney carcinoma; lane 8, SW480 colon carcinoma; lane 9, EJ bladder carcinoma.

Figure 5. Location of trapped exons in JJR-1 cDNA. The blackened box in the cDNA indicates open reading frame; a thin line indicates both 5' and 3' untranslated regions. Probe names correspond to those used in Figures 5A and B. Probe II (5A) represents bp 818-893 of JJR-1 and is overlapped by another trapped product. These overlapping products may have arisen from partial digestion of the cosmid or alternative splicing in COS7 cells occurring during exon amplification analysis.

Figure 5A (probe I) and Figure 5B (probe II) . Southern blot analysis of GUS5069 X hamster hybrid cell lines harboring the altered chromosome 22. DNA samples were digested with BamHI, size-fractionated in agarose gels, blotted and hybridized with radiolabeled probes indicated above each panel. Lane 1, GUS1323 (human control), lane 2, CHTG49 (hamster control); lane 3, (GUSH134A3 (hybrid with deleted homolog); lane 4, GUSH134B1 (hybrid with deleted homolog); lane 5, GM10888 (chromosome 22-only hybrid); lane 6, Eye3A6 (hybrid containing chromosome 22 and smaller portions of two to three other chromosomes).

Figure 5C (probe III), Figure 5D (probe IV) and Figure 5E (probe V) Southern blot analysis of NF2 patient lymphoblast DNA. Samples were digested with EcoRI, size-fractionated, blotted and hybridized with radiolabeled probes indicated above each panel. Lane 1, GUS1323 (human control); lane 2, GUS5069 (NF2 affected); lane 3, GUS5722 (NF2 affected) Probe V is comprised of multiple exons and recognizes multiple genomic fragments.

Figure [illegible] RT-PCR analysis of [illegible] described in Experimental Procedures. Samples were subjected to electrophoresis in a 1.5 % agarose gel and visualized by staining with ethidium bromide and ultraviolet illumination. The products seen correspond to the RNA

sequence encompassed by bases 1258 to 1947 (Primer pair:

5'AGGAGGCTGAACGCACGAG3' [SEQ ID No: 5] and
5'TGGTATTGTGCTTGCTGCTG3' [SEQ ID No: 6].

Lanes 1, 2, 4, lymphoblasts from independent NF2 patients: lane 3, GUS5068, lane 5, normal control lymphoblast; lane 6, GUS5722.

Figures 6A and 6B. SSCP analysis of RNA samples derived from primary cultures of meningioma tumors from NF2 patients. Lane 1, undenatured PCR product from the tumor in lane 2: lanes 2, 3, 4, and 5, denatured PCR product from independent primary NF2 meningiomas; lane 6, denatured PCR product from GUS5068 lymphoblast. The PCR reactions amplified the following segments: Figure 6A. bp 1709-1947 (Primer pair:

5'CTTCAACCTGATTGGTGACAG3' [SEQ ID No: 7] and
5'TGGTATTGTGCTTGCTGGTG3' [SEQ ID No: 8]

and Figure 6B: bp 457-730 (Primer pair:

5'AGGTACTGGATCATGATGTTTC3' [SEQ ID No: 9] and
5'TTTGGAAGCAATTCCTCTTGG3') [SEQ ID No: 10].

All lanes contain a proportion of undenatured product, detected by comparison with lane 1.

Figure 7A and 7B. Comparison of sequence identities in *merlin*-related proteins. The program PILEUP of the GCG package (Devereux, J. *et al.*, *Nucleic Acids Res. 12:387-395* (1984)) was used to generate an optimal alignment of the protein products translated from the following GenBank files: Human Moesin (M69066); Human Ezrin (X51521); Mouse Radixin (S66820); *Echinococcus multilocularis* tegument protein (M61186); human erythrocyte protein 4.1 (M14993) . Only amino acid identities are shown; non-identical residues are indicated by "." and gaps introduced by the program are represented by empty spaces.

Figure 8. Pedigree of family with neurofibromatosis type 2.

Only a portion of the large NF2 family described in detail previously (Wertelecki. W. *et al.*, *New Engl. J. Med. 319*:278-283 (1988)) is presented here schematically. Affected individuals are shown by a filled symbol. The sex for each individual in generations III through VI is not given to protect confidentiality. Diamond symbols indicate the presence of one or more additional offspring in a sibship, with no indication of NF2 state being presented, again to disguise the pedigree and provide confidentiality. The deduced genotypes for selected individuals are shown below the corresponding symbol as "1 1" (homozygous for the normal Asn220 codon) and "1 2" (heterozygous with one normal Asn220 codon, and one mutated Tyr220 codon). The arrow indicates the affected individual used in the initial screen to detect the putative mutation.

Figure 9. SSCP analysis of affected and unaffected individuals.

A non-denatured sample (ND) was run to identify the mobility of residual double stranded PCR product. Bands of abnormal mobility appear in lanes containing DNA derived from affected individuals and are identified by a small arrowhead. Samples represent paired sibs, with the odd numbered samples being from unaffecteds, and the even numbered samples deriving from affecteds.

Figures 10A and 10B. Direct DNA sequence analysis of the alteration.

The DNA sequence surrounding the site of the putative mutation is shown for both a patient (Fig. 10A) and normal control sample (Fig. 10B) with the corresponding protein sequence aligned by codon. The patient displays both an A and a T residue as the first base of codon 220 indicating the presence of a Tyr codon in addition to the normal Asn codon.

Figure 11. RsaI digestion to confirm the presence of the Asn220/Tyr mutation in affected individuals.

The amplified PCR products containing exon 7 from affected (A) and unaffected (U) individuals were digested

[illegible] agarose [illegible] Bakem agarose [illegible] products [illegible] products from affected individuals yield two fragments of 96 bp and 70 bp due to digestion of a RsaI site at crossing codons 218-219. PCR products from affected individuals produce an additional fragment of 67 bp due to digestion of the new RsaI

site at codons 219-220.

Figure 12. Conserved sequence domain affected by the putative mutation.

The amino acid sequence from residues 200-240 of the *merlin* protein is shown relative to the homologous regions for human moesin, human ezrin and mouse radixin (Trofatter, J. *et al.*, *Cell 72*:791-800 (1993)). The Asn residue (N) altered to a Tyr by the putative NF2 mutation is starred.

Figures 13A to 13C. Representative SSCP analyses.

For each exon, control blood (B) and tumor (T) DNAs were analyzed and a nondenatured sample (ND) was included. Abnormal migration patterns are observed for each tumor DNA. Two blood DNAs from NF2 patients, S11 and S33, show the same alterations in E2 as the tumor DNAs, suggesting that these shifts represent germline mutations.

Figures 14A and 14B. Direct DNA sequence analysis of two alterations in S29.

The DNA sequences surrounding the sites of two mutations are shown for tumor DNA (T) and control blood DNA (B) from patient S29. The tumor is heterozygous for each of a one base pair deletion in exon 2 and a four base pair deletion in exon 8 (shown in boxes) These deletions are predicted to generate premature stop codons at positions 123 and 251, respectively.

Figures 15A through 15Q. Exon sequences in the *merlin* gene with intron boundary sequences.

15A: Exon 1.
15B: Exon 2.
15C: Exon 3.
15D: Exon 4.
15E: Exon 5.
15F: Exon 6.
15G: Exon 7.
15H: Exon 8.
15I: Exon 9.
15J: Exon 10.
15K: Exon 11.
15L: Exon 12.
15M: Exon 13.
15N: Exon 14.
15O: Exon 15.
15P: Exon 16.
15Q: Exon 17.

[0008] The vertical line on the left indicates the 5' intron exon boundary. The vertical line on the right indicates the intron exon 3' boundary. The designation "N" indicates that the nucleotide is not definitively determined.

Summary of the Invention

[0009] The invention is directed to the protein *merlin*, mutants thereof, nucleic acid encoding this protein, nucleic acid encoding *merlin* regulatory regions and exons, mutant nucleic acid sequences, and uses thereof.

[0010] Accordingly, in a first embodiment, the invention is directed to purified preparations of the protein *merlin*, or mutants thereof.

[0011] In a further embodiment, the invention is directed to an isolated nucleic acid sequence encoding *merlin*, or mutants thereof.

[0012] In a further embodiment, the invention is directed to a recombinant construct containing nucleic acid encoding *merlin*, or mutants thereof.

[0013] In a further embodiment, the invention is directed to a vector containing nucleic acid encoding *merlin*, or mutants thereof.

[0014] [illegible]

[0015] [illegible]

[0016] In a further embodiment, the invention is directed to a method for diagnosing a merlin-associated tumor

such tumor being a tumor characterized by a loss, alteration, or decrease of the activity of the *merlin* tumor suppressor in the cells of said tumor, and especially by a loss or mutation of the *merlin* gene, in such cells. In one specific embodiment, the mutation is a change from A → T in the first position (base) of the amino acid at position 220 according to Figure 3, especially wherein tyrosine is substituted for asparagine at amino acid position 220. The nucleic acid change can be detected by *Rsa*I. In more general embodiments, the mutations include the genetic sequence alterations in the *merlin* gene, described in Example 6 herein, and which contribute to tumor formation and especially NF2.

[0017] In a further embodiment, the invention is directed to a method for treating a *merlin*-associated tumor in a patient, where the growth of such tumor reflects a functional change in *merlin*, a decreased level, or lack of *merlin* tumor suppressor activity in the tumor cell, the method comprising providing a functional *merlin* gene to the tumor cells of the patient, in a manner that permits the expression of the *merlin* protein provided by the gene, for a time and in a quantity sufficient to inhibit the growth of the tumor in the patient.

[0018] In a further embodiment, the invention is directed to a method of gene therapy of a symptomatic or presymptomatic patient, the method comprising providing a functional *merlin* gene to the relevant cells of the patient, both normal or tumor, in need of the therapy, in a manner that permits the expression of the *merlin* protein provided by the gene, for a time and in a quantity sufficient to provide the tumor suppressor function of *merlin* to the cells of the patient.

[0019] In a further embodiment, the invention is directed to a method for diagnosing NF2, the method comprising detecting a mutation in, or loss of, the *merlin* gene, or *merlin* protein, in a sample of non-tumor biological material from the subject to be diagnosed. This includes but is not limited to patients and single cells, such as embryonic cells or prenatal cells from amniotic fluid. In a specific embodiment, the detection is of a mutant *merlin* protein encoded by a mutation of A → T at the first position (base) of amino acid 220, especially where tyrosine has been substituted for asparagine at amino acid position 220, or of a mutated nucleotide coding sequence involving an A → T transversion at position the first base of amino acid 220, detectable by *Rsa*I digestion. In a more general embodiment, the detection is of a mutant *merlin* protein encoded by DNA containing mutations including those described in Example 6 herein and which produce an altered *merlin* protein.

[0020] In a further embodiment, the invention is directed to a method for screening an individual for future likelihood of developing *merlin*-associated tumors, or the disease NF2, the method comprising detecting a mutation in, or loss of, the *merlin* gene, or *merlin* protein, in a sample of biological material from the individual. In a specific embodiment, the detection is of a mutant *merlin* protein encoded by a mutation of A → T at the first position (base) of amino acid 220, especially where tyrosine has been substituted for asparagine at amino acid position 220, or of a mutated nucleotide coding sequence involving an A → T transversion at the first base of amino acid 220, detectable by *Rsa*I digestion. In more general embodiments, the detection is of a mutant *merlin* protein or mutant *merlin* DNA, wherein the mutation includes but is not limited to any of the mutations described herein, and that are clinically relevant.

[0021] In a further embodiment, the invention is directed to a method for treating NF2 in a patient, the method comprising providing a functional *merlin* gene to the desired cells of the patient, in a manner that permits the expression of the *merlin* protein provided by the gene, for a time and in a quantity sufficient to treat the patient.

[0022] In a preferred embodiment, a method is provided for identifying DNA sequence differences representing potential mutations within amplified coding sequences using single stranded conformational polymorphism analysis (SSCP). In this method, individual exons from the patient's DNA are first amplified by PCR. The amplification products are then denatured to separate the complementary strands and diluted to allow each single-stranded DNA molecule to assume a secondary structure conformation by folding on itself. Gel electrophoresis under non-denaturing conditions allows the detection of sequence changes compared to the normal (non-mutant) sequence.

[0023] In a further preferred embodiment, mutations discovered by application of the above method are used as standards of comparison for DNA from individuals suspected of being affected or known to be affected, so as to identify the mutation (if any) without full application of the above method. Knowledge of the exact mutation then allows the design of molecular therapeutic vehicles for gene therapy.

Detailed Description of the Preferred Embodiments

[0024] The *"merlin"* gene described herein is a gene found on human chromosome 22 that, as shown herein, contains non-overlapping and interstitial deletions in four independent NF2 patients and a single base change in affected individuals from five sibling pairs within an affected kindred, these results form the basis for concluding that this gene encodes a protein called *"merlin,"* which possesses a tumor suppressor activity. *merlin* has previously been termed "the *NF2* tumor suppressor." The *merlin* gene, therefore, includes the DNA sequences shown in Figures 3 and 15A-Q herein and all functional equivalents. The gene usually includes not only the coding sequences, but all regulatory

[illegible] addition, introns and other DNA sequences which are spliced from mRNA [illegible] transcript are [illegible] encompassed [illegible] and [illegible] encompassed [illegible] exonic and intronic sequences shown in Figures 15A-Q. Additionally, it is to be understood that the [illegible] gene

includes the corresponding genetic and functional sequences in non-human animal species.

[0025] The *merlin* gene of the invention encodes a novel protein, *merlin*, that is related to the moesin-ezrin-radixin family of cytoskeleton-associated proteins (Gould, K.L. *et al., EMBO J. 8*:4133-4142 (1989); Turunen, O. *et al., J. Biol. Chem. 264* 16727-16732 (1989); Funayama, N. *et al., J. Cell Biol. 115*:1039-1048 (1991); Lankes, W.T. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:8297-8301 (1991); Sato, N. *et al., J. Cell. Sci. 103*:131-143 (1992)). This protein, which is herein named *"merlin"* (moesin-ezrin-radixin-like protein), represents a new class of tumor suppressor whose function is mediated by interactions with the cytoskeleton. *Merlin* is found on human chromosome 22 between the known markers *D22S1* and *D22S28*.

I. Cloning Of Merlin DNA And Expression Of Merlin Protein

[0026] The identification of *merlin* cDNA and protein as the mutated or missing gene in tumors from NF2 patients is exemplified below. In addition to utilizing the exemplified methods and results for the identification of additional mutations or deletions of the *merlin* gene in NF2 patients, and for the isolation of the native human *merlin* gene, the sequence information presented in Figures 3 and 15A-Q represents nucleic acid and protein sequences, that, when inserted into a linear or circular recombinant nucleic acid construct such as a vector, and used to transform a host cell, will provide copies of *merlin* DNA and *merlin* protein that are useful sources for the *merlin* DNA and *merlin* protein for the methods of the invention. Such methods are known in the art and are briefly outlined below.

[0027] The process for genetically engineering the *merlin* coding sequence, for expression under a desired promoter, is facilitated through the cloning of genetic sequences which are capable of encoding the *merlin* protein. These cloning technologies can utilize techniques known in the art for construction of a DNA sequence encoding the *merlin* protein, such as polymerase chain reaction technologies utilizing the *merlin* sequence disclosed herein to isolate the *merlin* gene *de novo*, or polynucleotide synthesis methods to construct the nucleotide sequence using chemical methods. Expression of the cloned *merlin* DNA provides *merlin* protein.

[0028] As used herein, the term "genetic sequences" is intended to refer to a nucleic acid molecule (preferably DNA). Genetic sequences that are capable of being operably linked to DNA encoding *merlin* protein, so as to provide for its expression and maintenance in a host cell, are obtained from a variety of sources, including commercial sources, genomic DNA, cDNA, synthetic DNA, and combinations thereof. Since the genetic code is universal, it is to be expected that any DNA encoding the *merlin* amino acid sequence of the invention will be useful to express *merlin* protein in any host, including prokaryotic (bacterial) and eukaryotic (plants, mammals (especially human), insects, yeast, and especially cultured cell populations).

[0029] If it is desired to select a gene encoding *merlin de novo* from a library that is thought to contain a *merlin* gene, the library can be screened and the desired gene sequence identified by any means which specifically selects for a sequence coding for the *merlin* gene or expressed *merlin* protein such as, a) by hybridization (under stringent conditions for DNA:DNA hybridization) with an appropriate *merlin* DNA probe(s) containing a sequence specific for the DNA of this protein, the sequence being that provided in Figure 3 or a functional derivative thereof (that is, a shortened form that is of sufficient length to identify a clone containing the *merlin* gene), or b) by hybridization-selected translational analysis in which native *merlin* mRNA which hybridizes to the clone in question is translated *in vitro* and the translation products are further characterized for the presence of a biological activity of *merlin*, or, c) by immunoprecipitation of a translated *merlin* protein product from the host expressing the *merlin* protein.

[0030] When a human allele does not encode the identical sequence to that of Figures 3 or 15A-Q, it can be isolated and identified as being *merlin* DNA using the same techniques used herein. Many polymorphic probes useful in the fine localization of genes on chromosome 22 are known and available (see, for example, "ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries," fifth edition, 1991, pages 23-24; "Human gene mapping 10: tenth international workshop on human gene mapping," *Cytogenet. Cell Genet 51*:1-1148 (1989) and Rouleau, G.A. *et al.*, "A genetic linkage map of the long arm of human chromosome 22," *Genomics 4*:1-6 (1989)).

[0031] A useful *D22S1* probe is clone designation pMS3-18, a *Bgl*II-RFLP (allele 1: 8.2kb; allele 2: 3.6 kb); as described in Fontaine, B. *et al., Ann. Neurol. 29*:183-186 (1991) and Barker *et al., Cell 36*:131-138 (1984). An equivalent *D22S1* probe, pEDF139, is available from the ATCC (ATCC 59688 and ATCC 59689).

[0032] Other useful probes include: *D22S28* (clone W23C: ATCC 61636 and ATCC 61637); *D22S15; D22S32* (plasmid pEZF31: ATCC 50274 and ATCC 59275); *D22S42; D22S46; D22S56*, LIF (the leukemia inhibitory factor gene); and *NEFH* (the neurofilament heavy chain gene).

[0033] Human chromosome 22-specific libraries are known in the art and available from the ATCC for the isolation of probes ("ATCC/NIH Repository Catalogue of Human and Mouse DNA Probes and Libraries," fifth edition, 1991,

[illegible]

[0034] [illegible] not necessary to utilize the exact vector constructs exemplified in the invention. Equivalent vectors can be constructed using techniques known in the art. For example, the sequence of the [illegible] probe on plasmid pJL215

is published (see Figure 3 in Lees, J.L. *et al*, *EMBO J.* 7 1947-1955 (1988)). Since it is this sequence that provides the specificity for the *NEFH* gene, it is only necessary that a desired probe contain this sequence, or a portion thereof sufficient to provide a positive indication of the presence of the *NEFH* gene.

[0035] *Merlin* genomic DNA may or may not include naturally occurring introns. Moreover, the genomic DNA can be obtained in association with the native *merlin* 5' promoter region of the gene sequences and/or with the native *merlin* 3' transcriptional termination region.

[0036] *Merlin* genomic DNA can also be obtained in association with the genetic sequences which encode the 5' non-translated region of the *merlin* mRNA and/or with the genetic sequences which encode the *merlin* 3' non-translated region. To the extent that a host cell can recognize the transcriptional and/or translational regulatory signals associated with the expression of *merlin* mRNA and protein, then the 5' and/or 3' non-transcribed regions of the native *merlin* gene, and/or, the 5' and/or 3' non-translated regions of the *merlin* mRNA can be retained and employed for transcriptional and translational regulation.

[0037] Genomic DNA can be extracted and purified from any host cell, especially a human host cell possessing the long arm of chromosome 22, by means well known in the art. Genomic DNA can be shortened by means known in the art, such as physical shearing or restriction enzyme digestion, to isolate the desired *merlin* gene from a chromosomal region that otherwise would contain more information than necessary for the utilization of the *merlin* gene in the hosts of the invention. For example, restriction digestion can be utilized to cleave the full-length sequence at a desired location. Alternatively, or in addition, nucleases that cleave from the 3'-end of a DNA molecule can be used to digest a certain sequence to a shortened form, the desired length then being identified and purified by polymerase chain reaction technologies, gel electrophoresis, and DNA sequencing. Such nucleases include, but are not limited to, Exonuclease III and *Bal*31. Other nucleases are well known in the art.

[0038] Alternatively, if it is known that a certain host cell population expresses *merlin* protein, then cDNA techniques known in the art can be utilized to synthesize a cDNA copy of the *merlin* mRNA present in such population.

[0039] For cloning the genomic or cDNA nucleic acid that encodes the amino acid sequence of the *merlin* protein into a vector, the DNA preparation can be ligated into an appropriate vector. The DNA sequence encoding *merlin* protein can be inserted into a DNA vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are well known in the art.

[0040] When the *merlin* DNA coding sequence and an operably linked promoter are introduced into a recipient eukaryotic cell (preferably a human host cell) as a non-replicating, non-integrating, molecule, the expression of the encoded *merlin* protein can occur through the transient (nonstable) expression of the introduced sequence.

[0041] Preferably the coding sequence is introduced on a DNA molecule, such as a closed circular or linear molecule that is capable of autonomous replication. If integration into the host chromosome is desired, it is preferable to use a linear molecule. If stable maintenance of the *merlin* gene is desired on an extrachromosomal element, then it is preferable to use a circular plasmid form, with the appropriate plasmid element for autonomous replication in the desired host.

[0042] The desired gene construct, providing a gene coding for the *merlin* protein, and the necessary regulatory elements operably linked thereto, can be introduced into desired host cells by transformation, transfection, or any method capable of providing the construct to the host cell. A marker gene for the detection of a host cell that has accepted the *merlin* DNA can be on the same vector as the *merlin* DNA or on a separate construct for co-transformation with the *merlin* coding sequence construct into the host cell. The nature of the vector will depend on the host organism.

[0043] Suitable selection markers will depend upon the host cell. For example, the marker can provide biocide resistance, e.g., resistance to antibiotics, or heavy metals, such as copper, or the like.

[0044] Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0045] When it is desired to use *S. cerevisiae* as a host for a shuttle vector, preferred *S. cerevisiae* yeast plasmids include those containing the 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art and are commercially available.

[0046] Oligonucleotide probes specific for the *merlin* sequence can be used to identify clones to *merlin* and can be designed *de novo* from the knowledge of the amino acid sequence of the protein as provided herein in Figure 3 or from related proteins. Alternatively, antibodies raised against the *merlin* protein can be used to screen for the presence of unique protein determinants in transformants that express the desired cloned protein.

[0047] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a *merlin* protein if it includes

acid contains expression control sequences which contain transcriptional regulatory information and such sequences are "operably linked" to the *merlin* nucleotide sequence which encode the *merlin* polypeptide.

[0048] An operable linkage is a linkage in which a sequence is connected to a regulatory sequence (or sequences) in such a way as to place expression of the sequence under the influence or control of the regulatory sequence. If the two DNA sequences are a coding sequence and a promoter region sequence linked to the 5' end of the coding sequence, they are operably linked if induction of promoter function results in the transcription of mRNA encoding the desired protein and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the protein, antisense RNA, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter region is operably linked to a DNA sequence if the promoter is capable of effecting transcription of that DNA sequence.

[0049] The precise nature of the regulatory regions needed for gene expression can vary between species or cell types, but includes, as necessary, 5' non-transcribing and 5' non-translating (non-coding) sequences involved with initiation of transcription and translation respectively, such as the TATA box, capping sequence, CAAT sequence, and the like, with those elements necessary for the promoter sequence being provided by the promoters of the invention. Such transcriptional control sequences can also include enhancer sequences or upstream activator sequences, as desired.

[0050] The vectors of the invention can further comprise other operably linked regulatory elements such as DNA elements which confer antibiotic resistance, or origins of replication for maintenance of the vector in one or more host cells.

[0051] In another embodiment, especially for maintenance of the vectors of the invention in prokaryotic cells, or in yeast *S. cerevisiae* cells, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. In *Bacillus* hosts, integration of the desired DNA may be necessary.

[0052] Expression of a protein in eukaryotic hosts such as a human cell requires the use of regulatory regions functional in such hosts. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. Preferably, these regulatory signals are associated in their native state with a particular gene which is capable of a high level of expression in the specific host cell, such as a specific human tissue type. In eukaryotes, where transcription is not linked to translation, such control regions may or may not provide an initiator methionine (AUG) codon, depending on whether the cloned sequence contains such a methionine. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis in the host cell.

[0053] If desired, the non-transcribed and/or non-translated regions 3' to the sequence coding for the *merlin* protein can be obtained by the above-described cloning methods. The 3'-non-transcribed region of the native human *merlin* gene can be retained for its transcriptional termination regulatory sequence elements, or for those elements which direct polyadenylation in eukaryotic cells. Where the native expression control sequences signals do not function satisfactorily in a host cell, sequences functional in the host cell can be substituted.

[0054] It may be desired to construct a fusion product that contains a partial coding sequence (usually at the amino terminal end) of a first protein or small peptide and a second coding sequence (partial or complete) of the *merlin* protein at the carboxyl end. The coding sequence of the first protein can, for example, function as a signal sequence for secretion of the *merlin* protein from the host cell. Such first protein can also provide for tissue targeting or localization of the *merlin* protein if it is to be made in one cell type in a multicellular organism and delivered to another cell type in the same organism. Such fusion protein sequences can be designed with or without specific protease sites such that a desired peptide sequence is amenable to subsequent removal.

[0055] The expressed *merlin* protein can be isolated and purified from the medium of the host in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, affinity purification with anti-*merlin* antibody can be used. A protein having the amino acid sequence shown in Figure 3 can be made, or a shortened peptide of this sequence can be made, and used to raised antibodies using methods well known in the art. These antibodies can be used to affinity purify or quantitate *merlin* protein from any desired source.

[0056] If it is necessary to extract *merlin* protein from the intracellular regions of the host cells, the host cells can be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation.

[0057] It is to be understood that all of the above procedures that are applicable to cloning and expressing *merlin* sequences apply equally to normal and mutant sequences. Mutations may be in any of the regions, i.e., coding, non-[illegible] intronic, regulatory and the like.

[illegible] Mutations [illegible]

[0058] The definition of the sequence alteration predicted to change the sequence of the [illegible] protein in the

extended NF2 family originally employed to map the genetic defect on chromosome 22 is exemplified below (Wertelecki, W. *et al.*, *New Engl. J. Med. 319*:278-283 (1988); Narod, S.A. *et al.*, *Am. J. Hum. Genet. 51* 486-496 (1992)). The characterization of this mutation allows the early detection of the disorder in "at risk" family members before clinical symptoms appear. Although the exemplary material is directed to the detection of the disorder in a specific kindred, the exemplary methods can be applied to any family to define the precise molecular lesion underlying NF2, and this information can then be used to accurately determine whether at-risk members of the family have inherited the disorder.

[0059] Further, this information can be used to assess any given individual for the *merlin* genotype in that individual. Therefore, affected or other (e.g., carrier) individuals can be assayed for the presence of new mutations as well as for the presence of mutations that are previously defined. Accordingly, the exemplary material shows a preferred approach to isolating and characterizing the mutations associated with NF2 or other *merlin*-dependent pathologies.

[0060] New mutations in any desired kindred or affected individual or individual suspected of being affected can be defined using single-strand conformational polymorphism (SSCP) and sequence analysis of DNA amplified from the NF2 gene *merlin*. DNA alterations in the *merlin* coding sequence cause a shift on SSCP gels that is characteristic of the disease chromosome being transmitted with the disorder and present in only affected members of the pedigree. For example, as described below, an A → T transversion causes substitution of a tyrosine for an asparagine at position 220 of the *merlin* protein, in a region highly conserved in closely related members of the family of cytoskeletal-associated proteins. This alteration caused a shift on SSCP gels that was characteristic of the disease chromosome in this NF2 pedigree, being transmitted with the disorder, present only in affected members of the pedigree, absent in unaffected members of the family and absent from 158 unrelated individuals. Because of this identification, it is now possible to significantly alter the management of "at risk" members of this extended kindred, based on a precise definition of which individuals carry the disease gene, and which have escaped inheritance of the defect. A similar approach is applicable to defining the underlying lesion/DNA defect and thereby improving presymptomatic or prenatal diagnosis in any other NF2 family that does not have this specific mutation. As shown below in the exemplary material, an array of various mutations have been associated with the *merlin* locus. Accordingly, any of these relevant mutations may be used for purposes of diagnosis in specific kindreds or individuals.

[0061] Accordingly, a combination of amplification, for example using the polymerase chain reaction (PCR) with SSCP, permits the identification of other mutations in a *merlin* gene as simple as single base changes. Any regions of the *merlin* gene can be assessed by this procedure. The sequence now made available by the inventors allows the design of primers and amplification of any desired region including coding, intronic, and regulatory non-coding or non-transcribed.

[0062] The SSCP technique uses strategically placed primer sets to amplify small regions of the NF2 gene directly from genomic DNA using PCR. The double-stranded PCR product containing the amplified region can then be used as a template to generate single strands by priming multiple rounds of DNA synthesis with one of the oligonucleotides previously used in a double strand reaction. The amplified products can be used for SSCP analysis, cloned, and sequenced.

[0063] The amplification products, denatured to separate the complementary DNA strands, are diluted to allow each single-stranded DNA molecule to assume a secondary structure conformation by folding on itself. The single-stranded molecules are then subjected to electrophoresis, for example by polyacrylamide, under non-denaturing conditions. The secondary structure, which is highly dependent on the precise DNA sequence, affects mobility of the strand on the gel. Even a single base change or deletion can produce a visible shift in the final band position on the gel. Altered SSCP patterns thus can be analyzed based on a comparison with the SSCP patterns from affected family members, unrelated individuals, sporadic tumors, *et cetera*. The altered SSCP fragment pattern can then be correlated with inheritance of the disorder and associated with affected members of the pedigree.

[0064] Following SSCP analysis, the precise DNA alteration that causes the shifting mobility pattern can be ascertained by direct DNA sequencing of the PCR amplification product from which the altered single-stranded molecule was derived.

[0065] After a specific mutation is confirmed following PCR and SSCP analysis and direct sequencing, the presence of this mutation can be rapidly confirmed for any desired member of the kindred or otherwise by performing the same type of analysis.

[0066] Alternatively, an approach may be taken exploiting restriction enzyme digestion following PCR amplification in the area of the defined mutation. If the defined mutation creates a new restriction site, the presence of this mutation can be rapidly confirmed in other members of the kindred or otherwise by using primers in the area of mutation to amplify sequences that include the mutation and which, when subjected to restriction enzyme digestion, create restric-

[illegible] affected individuals may be subjected to electrophoresis and merely stained by ethidium bromide and [illegible] the resort to cumbersome radiolabeling or other labeling techniques.

[0067] In a specific disclosed embodiment, the genomic DNA of patients and unaffected control individuals was

amplified using primers in the intron flanking exon 7. SSCP analysis was then performed. The amplified products were also cloned and sequenced. Intron DNA sequence flanking each exon was determined. DNA primers that permit amplification of the exon sequences from genomic DNA using PCR were developed. A mobility shift was detected in the amplification products of exon 7 comprising base pair 819 to 894 of the *merlin* coding sequence. DNA sequencing was used to confirm the precise DNA alteration that caused the shifted mobility pattern in the SSCP analysis.

[0068] Normal *merlin* DNA displays an AAC codon encoding asparagine at position 220 of the *merlin* protein. The DNA with the shifted mobility reveals both an A and a T residue at the first position of this codon, suggesting a normal AAC codon on one chromosome and a mutated TAC on the other. The TAC would substitute a tyrosine at position 220.

[0069] The sequence change created a GTAC stretch that can be recognized by the restriction endonuclease *Rsa*I. The site created nine base pairs 3' to a preexisting *Rsa*I site in this exon. *Rsa* digests on the amplified PCR product confirm the presence of the same DNA change in other affected members of the family, its cotransmission with NF2, and its absence in unaffected members of the pedigree. Unaffected members display two *Rsa*I fragments of 96 base pairs and 76 base pairs, whereas the affected individuals produce an additional third fragment of 67 base pairs generated from the Rsa site created by the A → T change at codon 220.

[0070] Accordingly, in one embodiment of the invention, this specific mutation could be identified in individuals having or suspected of having NF2 by simple assays, e.g., Southern blots, involving restriction enzyme digestion with *Rsa*I. Similarly, if mutations in *merlin* create or abolish other restriction enzyme sites, this alteration can be exploited to recognize the mutation in individuals other than the one in whom the mutation was discovered by restriction enzyme cleavage assays.

[0071] In specific embodiments of the invention, SSCP analysis was performed to scan all seventeen exons of the entire *merlin* gene for mutations. In schwannomas from NF2 patients, base changes, deletions, and insertions were observed at various locations which resulted in missense, frameshift, and possible splice donor and splice acceptor alterations. The NF2 gene was also examined in sporadic schwannomas. Deletions, base changes, and insertions were observed in various locations in both intron and exon sequences. These mutations created frameshift, nonsense, and missense mutations, as well as actual or presumed alterations in the splice acceptor site, splice donor site, or acceptor branch site. Accordingly, in specific embodiments of the invention, these mutations may be useful as standards of comparison for the examination of at-risk individuals.

III. Use Of Merlin For Diagnostic And Treatment Purposes

[0072] It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses *merlin* and in which loss or mutation of *merlin* leads to pathological manifestations as in the human patient.

[0073] It is also to be understood that the methods referred to herein are applicable to any *merlin*-associated tumor or subject suspected of developing or having such a tumor, whether such tumor is sporadic or associated with a condition such as NF2.

[0074] A "*merlin*-associated" tumor is a tumor characterized in that the growth of such tumor reflects a decrease, functional alteration, or lack of *merlin* activity, especially if the decrease, change, or lack of *merlin* activity reflects a mutation or loss of the *merlin* gene or its regulatory regions. An example of such a tumor would include, but is not limited to, a schwannoma, (such as, for example, a vestibular schwannoma, and especially a bilateral vestibular schwannoma, a schwannoma of a cranial nerve, especially on the vestibular branch of the eighth cranial nerve, or a schwannoma of a spinal nerve root), or a meningioma (such as, for example, a meningioma of a cranial nerve, a vestibular meningioma, or a meningioma of a spinal nerve root).

[0075] The diagnostic and screening methods of the invention are especially useful for a person suspected of being at risk for developing *merlin*-associated tumor or disease and/or NF2 based on family history, or a person in which it is desired to diagnose or eliminate the presence of a *merlin*-associated condition or tumor or the NF2 condition as a causative agent behind a tumor growth.

[0076] By "predisposition to develop a *merlin*-associated tumor" is intended a genotype wherein a subject has the tendency to develop a genotype leading to the expression of an aberrant *merlin* gene. This could involve a subject heterozygous for a *merlin* mutation who subsequently becomes homozygous for the mutation such that the mutation is now expressed. Such a predisposition can be detected by molecular assays capable of detecting mutations or molecular changes in DNA, RNA, or *merlin* protein. Thus, even though a subject may be heterozygous for the *merlin* mutation, the mutation, if expressed on the affected chromosome, could then be detected, or, alternatively, the mutation in

[illegible] detected at the molecular level

[illegible] 575-578 (1988) Mulvihill [illegible] 39-52 (1990) specifically for NF2 the NIHCDCN diagnostic criteria are met if a person has either of the following:

1) Bilateral eighth nerve masses seen with appropriate imaging techniques (for example, computerized tomographic or magnetic resonance imaging),

2) A first-degree relative with NF2 and either unilateral eighth nerve mass or two of the following: neurofibroma, meningioma, glioma, schwannoma, and juvenile posterior subcapsular lenticular opacity.

[0078] According to the invention, presymptomatic screening of an individual in need of such screening is now possible using DNA encoding the *merlin* protein of the invention, and specifically, DNA having the sequence of the native human *merlin* gene. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of a missing or aberrant *merlin* gene in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed *merlin*-associated tumors and/or NF2. This is especially valuable for the identification of carriers of altered or missing *merlin* genes, for example, from individuals with a family history of *merlin*-associated tumors and/or NF2. This is also especially valuable for those patients where the chances of hearing preservation are optimal with early microsurgical removal of a vestibular schwannoma. Early diagnosis is also desired to maximize appropriate timely intervention as to any expected sequelae of the patient's tumor growth and lens opacities.

[0079] For example, in the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the 'normal' human *merlin* gene, (2) the presence of *merlin* mRNA and/or (3) the presence of *merlin* protein. The normal human gene can be characterized based upon, for example, detection of restriction digestion patterns in 'normal' versus the patient's DNA, including RFLP analysis, using DNA probes prepared against the *merlin* sequence (or a functional fragment thereof) taught in the invention. Similarly, *merlin* mRNA can be characterized and compared to normal *merlin* mRNA (a) levels and/or (b) size as found in a human population not at risk of developing *merlin*-associated tumors and/or NF2 using similar probes. Lastly, *merlin* protein can be (a) detected and/or (b) quantitated using a biological assay for *merlin* activity (its ability to suppress tumor growth) or using an immunological assay and anti-*merlin* antibodies. When assaying *merlin* protein, the immunological assay is preferred for its speed.

[0080] An (1) aberrant *merlin* DNA size pattern or sequence, and/or (2) aberrant *merlin* mRNA size, level, or sequence, and/or (3) aberrant *merlin* protein or level thereof would indicate that the patient is at risk for developing a *merlin*-associated tumor and/or NF2 and is likely to develop a *merlin*-associated tumor and/or NF2.

[0081] Similarly, if the tissue sample was derived from a tumor taken from a patient suspected of having a *merlin*-associated tumor and/or NF2, then (1) aberrant *merlin* DNA size, pattern, or sequence, and/or (2) aberrant *merlin* mRNA size, sequence, or level and/or (3) aberrant *merlin* protein or levels thereof would indicate that the patient has developed a *merlin*-associated tumor and/or NF2. These tumors can be treated with the methods of the invention as described below.

[0082] In accordance with the inventors' characterization of a specific *merlin* mutation in a particular kindred, and the guidance provided to extend the knowledge and approaches disclosed herein to the identification of other mutations and the identification of the mutation disclosed herein in other kindreds, preferred methods of screening tissue samples from presymptomatic, asymptomatic, or symptomatic individuals involve, rather than comparison with normal *merlin* protein, DNA, or RNA, direct detection of abnormal *merlin* genes and gene products.

[0083] Accordingly, a preferred strategy for identifying DNA sequences representing potential mutations within amplified coding sequences is the use of SSCP analysis combined with amplification and DNA sequencing. Accordingly, screening individual exons from a subject's DNA that have been amplified by PCR is a first approach. SSCP followed by direct DNA sequencing is then performed. In more preferred embodiments, mutations previously identified using this protocol provide a standard for comparison of the tissue sample to be assayed. The sample is thus amplified using primers known to be adjacent to the mutation and the amplification product either subjected to SSCP or subjected to restriction enzyme analysis in the case wherein the mutation creates or abolishes restriction sites found in the normal *merlin* gene.

[0084] Similarly, mutant mRNAs may also be the basis for assay of a subject's RNA in Northern blots where the abnormal RNA has a characteristic pattern as in electrophoresis. Alternatively, cDNA transcripts from the RNA of a subject can be analyzed by comparing such transcripts to the transcripts from known mutant *merlin* genes.

[0085] Alternatively, aberrant *merlin* proteins with characterized mutations may serve as the basis for comparison with proteins derived from the individual undergoing the diagnostic treatment. Thus, recognition by monoclonal or polyclonal antibodies using standard immunological assays may reveal the presence of mutated proteins that can be identified by comparison with previous mutations. Alternatively, methods of identifying mutant proteins using known mutants as comparisons include, but are not limited to, tryptic peptide digests.

[0086] Accordingly, a repository of mutant DNA, RNA/cDNA and protein patterns gathered from an analysis of the

[illegible]

[0087] [illegible] screening and diagnostic methods of the invention do not require that [illegible] coding sequence be used as a probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient

to detect the presence of the *merlin* gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern).

[0088] Prenatal diagnosis can be performed when desired, using any known method to obtain fetal cells, including amniocentesis, chorionic villous sampling, and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of chromosome 22 possessing the normal *merlin* gene is present in a heterozygous state.

[0089] The *merlin* DNA can be synthesized, and, if desired, labeled with a radioactive or nonradioactive reporter group, using techniques known in the art (for example, see Eckstein, F., ed., *Oligonucleotides and Analogues: A Practical Approach*, IRS Press at Oxford University Press, New York, 1992); and Kricka, L.J., ed., *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego, (1992)).

[0090] Although the method is specifically described for DNA-DNA probes, it is to be understood that RNA possessing the same sequence information as the DNA of the invention can be used when desired.

[0091] In the method of treating NF2 in a patient in need of such treatment, functional *merlin* DNA is provided to the cells of such patient, especially the tumor cells, in a manner and amount that permits the expression of the *merlin* protein provided by such gene, for a time and in a quantity sufficient to treat such patient. Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from the cell. For example, retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems, such as those described in U.S. Appl. No. 07/913,977 (filed July 16, 1992); U.S. Appl. No. 07/956,949 (filed October 6, 1992), U.S. Appl. No. 07/895,364 (filed June 9, 1992); each incorporated herein fully by reference. In addition, such methods are provided for, in, for example, the teachings of Breakefield, X.A. *et al.*, *The New Biologist 3*:203-218 (1991); Huang, Q. *et al.*, *Experimental Neurology 115*:303-316 (1992), WO93/03743 and WO90/09441 each incorporated herein fully by reference.

[0092] Delivery of a DNA sequence encoding a functional *merlin* protein, such as the amino acid encoding sequence of Figures 3 and 15A-15Q, will effectively replace the missing or mutated *merlin* gene of the invention, and inhibit, and/or stop and/or regress tumor growth that arose due to the loss of the *merlin* tumor suppressor.

[0093] This method is especially effective in the tumor types such as those classically associated with NF2, and especially with a schwannoma, (such as, for example, a bilateral vestibular schwannoma, a schwannoma of a cranial nerve, especially on the vestibular branch of the eighth cranial nerve, or a schwannoma of a spinal nerve root), meningiomas (such as, for example, a meningioma of a cranial nerve, a vestibular meningioma, or a meningioma of a spinal nerve root).

[0094] The method of the invention is also useful to treat conditions such as posterior capsular lens opacities, deafness, balance disorder, paralysis or other neurological problem when such problem is due to the presence of a *merlin*-associated tumor or NF2 condition.

[0095] The manner and method of carrying out the present invention can be more fully understood by those of skill by reference to the following examples, which examples are not intended in any manner to limit the scope of the present invention or of the claims directed thereto.

Examples

Experimental Procedure for Examples 1-4

NF2 Cell Lines

[0096] Lymphoblast cell lines were established (Anderson, M.A. *et al.*, *In Vitro 20*:856-858 (1984)) from affected members of NF2 pedigrees and from their unaffected relatives. Diagnosis of NF2 conformed to the criteria set forward by the National Institutes of Health Consensus Development Conference on Neurofibromatosis (Mulvihill, J.J. *et al.*, *Ann. Intern. Med. 113*:39-52 (1990)), except for the patient whose meningioma displayed a 4 bp deletion. This patient had a right vestibular schwannoma, and multiple meningiomas. Although she did not have a history of NF2, she probably represents a new mutation. Primary meningioma cells were cultured as described (Logan, J.A. *et al.*, *Cancer Genet. Cytogenet. 45*:41-47 (1990)) and analyzed after less than five passages.

[0097] Somatic cell hybrids were prepared by fusing GUS5069 lymphoblasts with a Chinese hamster cell line deficient in HPRT activity (CHTG49). Athwall, R.S. *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 74*:2943-2947 (1977)) using GIBCO PEG 4,000. Fused cell lines were selected by their ability to grow in media containing hypoxanthine, aminopterin and thymidine (HAT). Hybrids were screened for the chromosome 22 homologues using the polymorphic SSR marker, *TOP1P2* (Trofatter, J.A. *et al.*, *Hum. Mol. Genet. 1*:455 (1992)). Control hybrids GM10888 and Eve3FA6 (NA10027) are

DNA/RNA Blotting

[0098] DNA was prepared from cultured cells and DNA blots prepared and hybridized as described (Gusella, J.F *et al., Proc. Natl. Acad. Sci. U.S.A. 76*:5239-5243 (1979); Gusella, J.F. *et al., Nature 306*:234-238 (1983)). For pulsed-field gel analysis, agarose DNA plug preparation, and electrophoresis were carried out as described (Bucan, M. *et al., Genomics 6*:1-15 (1990)). RNA was prepared and Northern blotting performed as described in Buckler *et al.* Buckler, A.J. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:4005-4009 (1991).

Cosmid Walking

[0099] The *NEFH* probe used for blot analysis and to initiate cosmid walking was pJL215, representing a 4.4 kb KpnI/XbaI genomic fragment containing exon 4 and 3'UTR (Lees, J.F. *et al., EMBO J. 7*:1947-1955 (1988)). The *NEFH* probe pJL215 was obtained from Dr. Greg Elder and Dr. Robert Lazzarini, The Laboratory of Molecular Genetics, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD, 29892. Cosmid walking was performed in an arrayed cosmid library prepared from DNA of flow-sorted human chromosome 22 (LL22NC03); Dr. Pieter DeJong, Lawrence Livermore National Laboratory). Cosmid overlaps were identified by either hybridization of whole cosmid DNA or isolated fragments to filter replicas of the gridded arrays, or by PCR screening of row and column DNA pools. STSs were developed by direct cosmid sequencing using the T3 or T7 end-primers (McClatchey, A.I. *et al., Hum. Mol. Genet. 1* 521-527 (1992)).

cDNA Isolation and Characterization

[0100] Human frontal cortex and hippocampus cDNA libraries in lambdaZAPII (Stratagene) were screened using exon probes isolated and prepared as described by Buckler *et al.* (Buckler, A.J. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:4005-4009 (1991)). cDNA clones and trapped exon were sequenced as described (Sanger, T. *et al., Proc. Natl. Acad. Sci. U.S.A. 74*:5463-5467 (1977)). Direct PCR sequencing was performed as described (McClatchey, A.I. *et al., Cell 68*:769-774 (1992)). Screening for variations by SSCP analysis followed the procedure described in Ambrose *et al.* (Ambrose, G. *et al., Hum. Mol. Genet. 1*:697-703 (1992)). RNA was reverse transcribed using an oligo(dT) primer (BRL reverse transcriptase) to prepare first strand cDNA. Portions of the cDNA were amplified using the following primer sets:

5'CCAGCCAGCTCCCTATGGATG3' [SEQ ID No: 11] and
5'AGCTGAAATGGAATATCTGAAG3' [SEQ ID No: 12]

to amplify bp 824-2100 and

5'GCCTTCTCCTCCCTGGCCTG3' [SEQ ID No. 13] and
5'GATGGAGTTCAATTGCGAGATG3' [SEQ ID No: 14]

to amplify bp 314-1207. These cold PCR products were then reamplified with specific regional primers for SSCP as described in the legend to Figures 6, 6A and 6B.

Example 1

Scanning the NF2 Candidate Region for Rearrangement

[0101] The region on chromosome 22 that was examined for the presence of the NF2 gene was between *D22S1* and *D22S28*; this region was estimated to encompass 6 Mb of band q12 (Frazer, K.A. *et al., Genomics 14*:574-584 (1992)).

[0102] By scanning the human chromosome 22 region between *D22S1* and *D22S28* for loss of DNA it was determined whether some germline NF2 mutations might involve a deletion of the tumor suppressor gene as has been found in Wilms' tumor and retinoblastoma (Riccardi, V.M. *et al., Pediatrics 61*:604-610 (1978); Francke, U. *et al., Cytogenet. Cell Genet. 24*:185-192 (1979); Dryja, T.P. *et al., Proc. Natl. Acad. Sci. U.S.A. 83*:7391-7394 (1986)). Pulsed-field gel [illegible] containing lymphoblast DNA from [illegible] NF2 patients were probed for several loci in the candidate region [illegible] revealed that [illegible] lymphoblast [illegible] from a female NF2 patient, a probe for the [illegible] locus hybridized to apparently altered fragments of reduced size with both NotI and NruI.

[0103] In NotI-digested DNA (Figure 1), the *NEFH* probe detected fragments of approximately 600 kb, 400 kb and 230 kb in most lymphoblast cell lines. It was not possible to confirm that the 600 kb fragment originated from chromosome 22. Thus, it was possible that hybridization had occurred with a related locus (Menon *et al.*, unpublished results). Variable intensity of the 230 kb fragment in many samples suggested that it resulted from partial digestion of the 400 kb fragment. In GUS5069, additional fragments of approximately 370 kb and 200 kb were observed. These results are consistent with the possibility of a deletion within the region common to the 400 kb and 230 kb fragments. The alteration was transmitted along with NF2 from the patient to her affected daughter (represented by GUS5068) (Figure 1).

Example 2

Chromosome Walking Toward the Merlin Gene

[0104] To isolate DNA corresponding to the region of chromosome 22 apparently deleted in GUS5069, a bi-directional cosmid walk was initiated from *NEFH*. At each step, single restriction fragments of the cosmids were used as probes on pulsed-field gels to establish the location of the putative deletion relative to *NEFH*. On the 5' side of *NEFH*, a NotI site that was rarely cleaved in lymphoblast DNA was identified. Probes beyond the NotI site detected the same approximately 400 kb NotI fragment along with a 170 kb fragment of variable intensity. Thus, infrequent cleavage of this NotI site divides the 400 kb fragment into fragments of 230 kb and 170 kb. Since the putative deletion in GUS5069 affects the 230 kb fragment but not the 170 kb fragment, further experiments continued to walk only 3' of *NEFH*. Pulsed-field gel blots containing DNA from GUS5069 were again probed.

[0105] The NotI pulsed-field gel map and a minimal set of clones representing the cosmid walk and the extent of the genomic deletion (see Figure 5A) are shown in Figure 2. The deletion was reached when a probe (Figure 2, Probe A) was tested from cosmid 96C10 which failed to detect the altered NotI fragment in GUS5069. However, various probes from cosmid 28H6 and 121G10 did detect the altered fragment.

[0106] To estimate the extent of deletion, probes B and C (Figure 2) were tested. Probe B is an 8 kb HindIII fragment from 28H6 which overlaps with the T3 end of 96C10. Probe C is a 9 kb HindIII fragment from the T7 end of 96C10. Probe B detected both the normal and the altered NotI PFG fragment, but probe C detected only the normal fragment.

[0107] For more precise analysis of the deletion, the altered chromosome 22 from GUS5069 was segregated from its normal counterpart in human X hamster somatic cell hybrids. STS assays for the T3 and T7 ends of 96C10 were created and hybrids containing the separated chromosomes 22 were tested. In contrast to the above hybridization results, the T3 end of 96C10 was absent in hybrid GUSH134A3, containing the deleted chromosome but present in GUSH134A10 containing the normal chromosome. Moreover the T7 assay was positive in both hybrids. The locations of probes B and C, and of both STS assays were confirmed on the cosmid walk. Thus, the failure of probe C to detect the altered fragment suggests that the deletion spans most but not all of this sequence. Similarly, the other deletion breakpoint must occur within the region spanned by probe B. Therefore, the results of hybridization and PCR indicate that the deletion must encompass almost all of 96C10 and up to an additional 5 kb of 28H6. This 35-45 kb region is expanded below the cosmid walk in Figure 2.

Example 3

Identification and Characterization of the merlin cDNA

[0108] Exon amplification (Buckler, A.J. *et al., Proc. Natl. Acad. Sci. USA 88*:4005-4009 (1991)), which produces cloned "trapped exons," was applied to cosmids 28H6, 96C10, 121G10, 123F5, 10H11, and 7C4 surrounding the site of the NF2 deletion as a rapid method of obtaining exonic probes for cDNA cloning. Each exon clone can represent a single exon, or multiple exons spliced together in the trapping procedure. Twenty-four exon clones were obtained and sequenced, 6 of which displayed sequence similarity with the cytoskeleton-associated proteins moesin, ezrin and radixin (see below). The latter exons were used to screen human frontal cortex and hippocampus cDNA libraries.

[0109] Figures 3A to 3F show the complete DNA sequence of JJR-1, the longest clone obtained in cDNA screening. JJR-1 has been deposited with the American Type Culture Collection and assigned ATCC75509. This sequence contains eight of the cloned exon segments as shown in Figure 5. The cDNA is 2,257 bp long and shows no evidence of a poly(A) tail. However, two shorter cDNA clones, JJR-6 and JJR-9, which overlapped the restriction map of JJR-1, had apparent poly(A) tails beginning at base 2231. JJR-1 contains an open reading frame of 1785 bp, encoding a pre-[illegible] of 69 kD. The [illegible] 90 bp upstream of the putative initiator methionine. The [illegible]

[0110] Both the JJR-1 cDNA sequence and the predicted protein product were used to search for similarity in nucleic acid and protein databases using the BLAST network service of the National Center for Biotechnology Information (Alt-

schul, S.F. *et al., J. Mol. Biol. 215*:403-410 (1990)). The DNA sequence displayed significant similarity to moesin and ezrin genes from several species, including man ($P=9.0e^{-125}$ and $9.0e^{-122}$), to mouse radixin ($1.1e^{-102}$) and to *Echinococcus multilocularis* tegument protein ($2.4e^{-21}$). Striking similarity was also detected at the amino acid level with these same proteins ($2.5e^{-146}$, $5.0e^{-146}$, $2.7e^{-145}$ and 7.6^{-73}, respectively) and to a potential product of a sequence tag from *Caenorhabditis elegans* ($3.7e^{-43}$). Weaker similarities were detected to the sequences of two protein tyrosine phosphatases, PTP-MEG and PTP-H1 ($1.3e^{-17}$ and 9.6^{-16}, respectively), to erythrocyte protein 4.1 ($9.9e^{-14}$) and to a wide range of myosin, tropomyosin and paramyosin proteins. Because this novel gene is most closely related to moesin, ezrin and radixin (45-47% identity), it is called "*merlin*."

[0111] Northern blot analysis using total RNA from various cultured human tumor cell lines (Figure 4) revealed two major hybridizing species of 2.6 kb and 7 kb, and a less intensely hybridizing RNA of 4.4 kb. A similar pattern was detected in poly(A) + RNA from various human tissues, including heart, brain, lung, skeletal muscle, kidney, pancreas and weakly in liver indicating that the *merlin* gene is expressed widely. The apparent poly(A) tails detected in JJR-6 and JJR-9 suggest that these clones may have derived from the approximately 2.6 kb RNA. The JJR-1 clone likely derived from one of the larger RNAs which apparently has a much longer 3' UTR. However, it cannot be excluded that the larger RNAs arise by alternative splicing that alters the length and composition of the coding sequence or by hybridization to related family members.

Example 4

Non-Overlapping Deletions Interrupt the Candidate NF2 Gene

[0112] To determine whether the deletion detected in GUS5069 interrupts the *merlin* gene, exon probes were prepared from across the coding sequence (Figure 5) and Southern blots containing DNA from GUSH134A3 and GUSH134B1 (two independent hybrid lines containing the deleted chromosome 22) were analyzed. The results for probes I and II, shown in Figure 5A and Figure 5B respectively, demonstrate that the probe I sequence was absent from both hybrids, while the probe II sequence was present in both. Thus, she genomic deletion truncates the *merlin* gene within the coding sequence between probes I and II, removing the 5' end.

[0113] In a search for additional alterations in the *merlin* gene, blots of restriction-digested DNA from 33 unrelated NF2 patients were scanned using the cDNA as probe. One patient, represented by cell line GUS5722, displayed altered fragments with several restriction enzymes suggestive of a small ~3.4 kb genomic deletion. This patient was analyzed using Southern blotting as shown in Figures 5C to 5E. Probes III, IV and V all reside on the same 21 kb EcoRI fragment. In GUS5722, probes III and V detected both the normal EcoRI fragment and second fragment reduced in size by deletion. Probe IV failed to detect the altered fragment in GUS5722 because it lies within the region deleted. PCR amplification of first strand cDNA from GUS5722 was performed and confirmed the presence of two types of PCR product (Figure 6). Direct sequencing revealed that the novel PCR product was missing bases 1559 to 1792 of the cDNA, representing deletion of at least two exons. The absence of this segment would remove 78 amino acids from the protein, while leaving the reading frame intact. The GUS5722 cell line was Generated from a member of a large NF2 kindred (Family 3 in Narod, S.A. *et al., Am. J. Hum. Genet. 51*:486-496 (1992)), and the deletion was present in five affected members and absent in eleven unaffected members of this pedigree.

[0114] The presence of non-overlapping deletions affecting the *merlin* gene in two independent families supported the conclusion that this gene represents the NF2 tumor suppressor. The presence of additional alterations were determined by single-strand conformational polymorphism (SSCP) analysis of PCR amplified first strand cDNA from tumor and lymphoblast samples. mRNA from four primary cultures of meningiomas (3 from NF2 patients with a family history of the disorder, 1 from a probable new mutation to NF2) was used and only selective regions of the mRNA were analyzed. Two of the tumors yielded aberrant patterns.

[0115] A meningioma from a female patient likely to have NF2 (see Experimental Procedures) displayed a reduced size for the expected non-denatured PCR product on SSCP gels (Figure 6A). The vastly reduced level of the normal-sized PCR product suggests that this tumor had lost alleles in this region of chromosome 22. However, lymphocyte DNA was not available from this patient to confirm this. Direct sequence analysis of the PCR product confirmed the presence of a 4 bp deletion which removes bases 1781 to 1784. This deletion alters the reading frame and generates a shorter protein.

[0116] A meningioma from a male patient with NF2 (see Experimental Procedures) displayed an altered pattern on SSCP analysis (Figure 6B). This meningioma was known to have lost heterozygosity on chromosome 22 based on [illegible] of polymorphic markers in blood and tumor DNA. Thus, the tumor suppressor model would suggest that [illegible] matically alters the predicted protein by introducing a stop codon within 100 bases

Discussion

[0117] The delineation of non-overlapping deletions affecting different portions of the same chromosome 22 gene in two independent NF2 families is strong evidence that *"merlin"* is the NF2 tumor suppressor. Although it is possible that one or both of these deletions may affect a second gene in the area, should this gene in fact be the *NF2* tumor suppressor, it would have to be affected by both deletions and must therefore be composed of exons interspersed with those of the *merlin* gene.

[0118] The larger of the deletions truncates the 5' end of the *merlin* gene, removing at least 120 amino acids. In addition, the extent of this deletion suggests that the 5' regulatory elements may also be missing. The smaller germline deletion removes 78 amino acids from the C-terminal portion of the protein. It is likely that such alterations would have drastic consequences for the function of the *merlin* protein.

[0119] The four base pairs and single base pair deletions in meningiomas from unrelated NF2 patients could possibly be of somatic origin and unrelated to the inherited predisposition. However, the almost exclusive expression of the altered copy of the *merlin* gene suggests that the normal sequence has been lost as a somatic event in tumor formation. This is consistent with the tumor suppressor model, and would suggest that the frameshift alterations actually represent germline mutations in these patients.

[0120] The *merlin* protein encoded at the candidate *NF2* locus is a novel member of a growing family of proteins that have been proposed to act as links between the cell membrane and the cytoskeleton (Luna, E.J. *et al., Science 258*:955-964 (1992); Sato, N. *et al., J. Cell. Sci. 103*:131-143 (1992)). All members of the family (which includes moesin, ezrin, radixin, erythrocyte protein 4.1 and talin) contain a homologous domain of approximately 200 amino acids near the N-terminus followed by a segment that is predicted to be rich in a α-helix structure, and a highly charged C-terminal domain. Where they have been characterized from more than one mammalian species, members of this family are remarkably conserved. Moreover, highly related genes have been detected in the nematode, *Caenorhabditis elegans* (Waterson, R. *et al., Nature Genet. 1*:114-123 (1992)), and in the parasitic cestode, *Echinococcus multilocularis* (Frosch, P.M. *et al., Mol. Biochem. Parasitol. 48*:121-130).

[0121] Although most distantly related to *merlin*, protein 4.1 and talin are the best studied members of this family of proteins and have contributed the most towards understanding the function of the gene family. Protein 4.1 plays a critical role in maintaining membrane stability and cell shape in the erythrocyte by connecting the integral membrane proteins glycophorin and protein 3 (the anion channel) to the spectrin-actin lattice of the cytoskeleton (Leto, T.L. *et al., J. Biol. Chem. 259*:4603-4608 (1984); Conboy, J. *et al., Proc. Natl. Acad. Sci. U.S.A. 83*:9512-9516 (1986)) Genetic defects in protein 4.1 lead to one form of hereditary elliptocytosis (Tchernia, G. *et al., J. Clin. Invest. 68*:454-460 (1981); Delaunay, J. *et al., Nucleic Acids Res. 12*:387-395 (1984)). The binding site for glycophorin in protein 4.1 has been mapped to the N-terminal domain, suggesting that the homologous region in other family members might also bind to proteins in the membrane (Leto, T.L. *et al.* in *Membrane Skeletons and Cytoskeletal Membrane Associations,* Bennett *et al.*, eds. Liss, New York (1986), pp. 201-209). Interestingly, a related domain is also found in two protein tyrosine phosphatases, PTP-MEG and PTP-H1, perhaps allowing these enzymes to associate with the membrane or the cytoskeleton (Gu, M. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:5867-5871 (1991); Yang, Q. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:5949-5953 (1991)). Binding of protein 4.1 to spectrin is mediated by the α-helical region of the protein, suggesting that the analogous segments of the other family members might also bind to cytoskeletal components (Correas, I. *et al., J. Biol. Chem. 261*:3310-3315 (1986)). Talin, a large protein found in regions of focal adhesions at cell-cell or cell-substrate contacts, appears to behave similarly, binding to the integrins in the cell membrane and to vinculin, thereby connecting the extracellular adhesion matrix to the cytoskeleton (Rees, D.J.G. *et al., Nature 347*:685-689 (1990), Luna, E.J. *et al., Science 258*:955-964 (1992)).

[0122] Moesin, ezrin and radixin are highly related proteins (~ 70-75 % amino acid identity) that have each been postulated to provide a link between the cytoskeleton and the cell membrane. Each of these proteins shares 45-47% amino acid identity with *merlin*. Moesin (membrane-organizing extension spike protein), originally proposed as a receptor for heparin sulfate, has been found at or near the membrane in filopodia and other cell surface protrusions (Lankes, W.T. *et al., Proc. Natl. Acad. Sci. U.S.A. 88*:8297-8301 (1991); Furthmayr, H. *et al., Kidney Int. 41*:665-670 (1992)). Ezrin (cytovillin) has been seen in association with microvilli and cellular protrusions in many cell types (Pakkanen, R. *et al., J. Cell. Biochem. 38*:65-75 (1988); Gould, K.L. *et al., EMBO J. 8*:4133-4142 (1989); Turunen, O. *et al., J. Biol. Chem 264*:16727-16732 (1989); Hanzel, D. *et al., EMBO J. 10*:2363-2373 (1991); Birbauer, E. *et al., J. Neurosci. Res. 30*:232-241 (1989)) Rapid redistribution of ezrin to regions of membrane remodeling, such as microvillar formation and membrane ruffling in response to growth factor stimulation, may be regulated by phosphorylation of the protein on both

[illegible] (Pietscher A [illegible] Biol 18:921-930(1988) Kreg [illegible] J Biol Chem 267:19258-

[illegible] 454-4[illegible] navam-

[illegible] 1039-1048(1991) Interestingly in mitotic cells radixin is concentrated at the cleavage furrow (Sato, N. *et al., J. Cell Biol. 115*:321-320 (1991);

[0123] *Merlin* possesses an N-terminal domain that is similar to protein 4.1 (28% identity), and to talin (21% identity). It is much more closely related, however, to moesin, ezrin and radixin (Figure 7). Amino acid identity between *merlin* and the three latter proteins is concentrated in the first 342 residues (~63% identity). Like these other family members, the *merlin* protein is predicted to have a very long α-helical domain spanning 160-170 amino acids, beginning around residue 300. The first third of this domain overlaps with the region of strongest homology to moesin, ezrin and radixin. However, the remaining stretch shows limited similarity with these proteins and with a wide variety of myosins and tropomyosins. The C-terminal region of *merlin* contains a hydrophilic domain analogous to those of other family members. The similarity in structure of *merlin* to the other members of this family suggests that it too may normally act as a link between the cytoskeleton and the cell membrane and may thus represent a new class of tumor suppressor gene.

[0124] The cytoskeleton of mammalian cells is a complicated lattice-work of many different kinds of interconnected filaments (Luna, E.J. *et al., Science 258*:955-964 (1992)). It participates in a wide range of crucial cellular activities, including determining and altering shape, movement, cell division, cell-cell communication, cell anchorage, and organization of the intracellular milieu (Bernal, S.D. *et al., Crit. Rev. Oncol. Hematol 3*:191-204 (1985)). A defect in a protein which connects some component of this network to the plasma membrane could affect any of these processes, and have a consequent effect on growth control. For example, inactivation of the *merlin* protein may disregulate growth by disrupting a signal transduction pathway, by altering anchorage dependence, by upsetting the cell cycle regulation, or by some other mechanism remains to be determined. However, the characteristic structure of the *merlin* protein suggests that a search for its membrane and cytoskeletal binding targets might provide a logical route to exploring this question.

Example 5

Altered Coding Sequence of the Merlin Tumor Suppressor Permitting DNA Diagnosis in an Extended Pedigree with NF2

[0125] The objective of this example was to define the DNA mutation causing NF2 in a large, well-studied NF2 pedigree previously used to chromosomally map and to isolate the disease gene. The design was to use SSCP and sequence analysis of DNA amplified from the NF2 gene of affected and unaffected persons. The participants in the study set forth in this example were affected, unaffected, and at-risk members of a large pedigree segregating NF2. The results of the study showed a DNA alteration in the *merlin* coding sequence causing a shift on SSCP gels that was characteristic of the disease chromosome in this NF2 pedigree, being transmitted with the disorder, present only in affected members of the pedigree, absent in unaffected members of the family, and absent from 158 unrelated individuals. The alteration caused substitution of a tyrosine for an asparagine at position 220 of the *merlin* protein, in a region highly conserved in closely-related members of the family of cytoskeletal-associated proteins. The DNA change could also be detected by restriction enzyme digestion with *Rsa*I.

A. Materials and Methods

1. Patients

[0126] The family studied in this Example has been extensively characterized clinically and described in detail previously (Wertelecki, W., *et al., New Engl. J. Med. 319*:278-283 (1988)). Medical records, histologic slides, death certificates and autopsy reports were sought for all symptomatic family members. Clinical assessments were performed including a search for signs of neurofibromatosis type 1. The results of computed tomography, MRI, and ophthalmologic and audiologic examinations were also sought. Diagnostic criteria used were those of the NIH consensus statement on neurofibromatosis (National Institutes of Health Consensus Development Conference Statement: Neurofibromatosis *Arch Neurol. 45*:575-578 (1988)). Lymphoblast lines were established from peripheral blood samples for all patients and relatives as previously described (Anderson *et al., In Vitro 20*:856-858 (1984)). DNA was isolated from peripheral or cultured leukocytes as described herein.

2. Polymerase Chain Reaction (PCR) Amplification of Exon 7

[0127] [illegible] DNA [illegible] and the unaffected control individuals were amplified using primers in the [illegible] amplification [illegible] reduce [illegible] each [illegible] dGTP, dCTP and dTTP, 4 pmoles of each primer, 0.5 units *Taq* polymerase, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1 mg/ml gelatin in a total volume of 10 µl. Each reaction was cycled 35 times using the following steps: denat

turation (at 95°C for 1 5 minutes), primer annealing (at 60°C for 1.5 minutes), and elongation (at 72°C for 30 seconds).

3. Single Strand Conformational Polymorphism Analysis (SSCP)

[0128] SSCP analysis was performed according to the procedure of Orita *et al.* with minor modifications (Orita, M., *et al., Genomics 5*:874-879 (1989)). PCR amplification was carried out as described above except each reaction included 0.1 µl (10 mCi/ml) of α-^{32}P dATP (Amersham Life Sciences). The amplified products were diluted 1:20 in 0.05% SDS, 6 mM EDTA, 40% formamide, 0.5 mg/ml xylene cyanol and 0.5 mg/ml bromphenol blue and heated to 90°C for 3 minutes to denature the DNA. Samples were immediately cooled on ice and loaded on an 8% polyacrylamide gel containing 8% glycerol. Electrophoresis was carried out at room temperature for 12 to 16 hours at a constant power of 6-10W. Gels were dried and exposed overnight to Kodak X-Omat AR film.

4. DNA Sequencing

[0129] The double-stranded PCR product containing the amplified exon was used as a template to generate single-strands by priming multiple rounds of DNA synthesis with one of the oligonucleotides previously used in the double-strand reaction (Gibbs, A., *et al., Proc. Natl. Acad. Sci. USA 86*:1919-1923 (1989)). Conditions for the single strand-producing reactions were identical to the PCR amplification of individual exons as described above. After 30 cycles, the product was ethanol precipitated in the presence of ammonium acetate and resuspended in 7 µl water for subsequent sequence analysis. The sequencing reactions were performed as described in Park *et al. (Hum. Mut. 1*:293-297 (1992)) utilizing Sequenase (US Biochemicals). The amplified products were also cloned in T vector (Novagen) and the DNA obtained from the individual clones were sequenced using the Sequenase kit (US Biochemicals).

B. Results

1. The Extended NF2 Pedigree

[0130] The NF2 gene shown in previous Examples was mapped precisely using genetic linkage analysis in an extremely large disease pedigree from which 137 blood samples were tested for polymorphic DNA markers on chromosome 22 (Wertelecki, W., *et al., New Engl. J. Med. 319*:278-283 (1988)). For this Example, a subset of the members of this kindred was selected to use for identification of the underlying NF2 mutation. The relationships of the family members used is shown in Figure 8.

2. Scanning for NF2 Mutation Affecting Expression of Merlin

[0131] The NF2 gene has been identified herein based on non-overlapping genomic DNA deletions that altered its coding sequence in three independent NF2 families and in a meningioma from an unrelated NF2 patient. The *merlin* mRNA sequence consists of more than 2250 bases. The translated portion of the mRNA, an open reading frame of 1785 bases encoding the predicted 595 amino acid *merlin* protein is bracketed by 5' and 3' untranslated regions whose extent and variability remain to be defined completely. The *merlin* mRNA is spliced together from at least 17 exons that are distributed across about 100 kb of chromosome 22. In order to scan for mutations in the NF2 gene, the intron DNA sequence flanking each exon was determined, and DNA primers that permit amplification of the exon sequences from genomic DNA using the PCR have been developed.

[0132] The strategy for identifying DNA sequence differences representing potential mutations within the amplified coding sequences is the use of SSCP analysis (Orita, M., *et al., Genomics 5*:874-879 (1989)). In this method, individual exons from the patient's DNA are first amplified by PCR. The amplification products are then denatured to separate the complementary DNA strands and diluted to allow each single stranded DNA molecule to assume a secondary structure conformation by folding on itself. The single stranded DNA molecules are then subjected to polyacrylamide gel electrophoresis under nondenaturing conditions. The secondary structure, which is highly dependent on the precise DNA sequence, affects mobility of the strand on the gel. Even a single base change or deletion can produce a visible shift in the final band position on the gel.

[0133] The SSCP technique was applied to the DNA samples from the family in Figure 8. A mobility shift was detected when comparing amplified PCR products from the affected family member, designated by an arrow in Figure 8, relative to those from several normal unrelated individuals and from members of other unrelated NF2 families. This alteration, characteristic of the disease allele and transmitted with the disorder, additional affected and unaffected members of the NF2 pedigree were typed. Figure 8 shows the deduced genotype of each family member tested in this and other analyses. Figure 9 displays the results of one SSCP analysis in which five sibling pairs, each consisting of

one affected and one unaffected individual, were tested. The altered SSCP pattern shows a clear correlation with inheritance of the disorder, and is present only in affected members or the pedigree. This SSCP pattern was not observed in a search of more than 300 independent chromosomes, derived from normal controls, other NF2 families, and sporadic tumors of various types (colon, astrocytoma, schwannoma etc.).

3. Identification of the DNA Sequence Alteration Underlying the SSCP Variation

[0134] To determine the precise DNA alteration that caused the shifted mobility pattern in the SSCP analysis, direct DNA sequencing of the PCR amplification product from an affected member of the pedigree and an unrelated normal control was performed. The change in DNA sequence in the affected individual and its absence in an unaffected member of the pedigree was reconfirmed using cloned PCR product. The results of sequence analysis are shown in Figures 10A and 10B.

[0135] The control sequence displays an AAC codon encoding asparagine at position 220 of the *merlin* protein. The DNA from the patient reveals both an A and a T residue at the first position of this codon, suggesting a normal AAC codon on one chromosome, and a mutated TAC on the other. The TAC codon would substitute a tyrosine at position 220. Since this SSCP variation was absent from more than 300 independent chromosomes, it is unlikely that this change is simply a polymorphism. Rather, the change from the aliphatic side chain of asparagine to the bulky aromatic side chain of the tyrosine is likely to have significant consequences for the structure of the protein.

4. Confirmation of the Sequence Alteration by RsaI Digestion

[0136] The sequence change outlined above creates a GTAC stretch that is recognized and cleaved by the restriction endonuclease RsaI. This site is created 9 bp 3' to a pre-existing RsaI site in this exon. Thus, to confirm the presence of the same DNA change in other affected members of the family, its cotransmission with NF2, and its absence in unaffected members of the pedigree, we performed RsaI digests on the amplified PCR product from several family members. Typical results are shown in Figure 11. The unaffected members of the family (U) and other control individuals display two RsaI fragments of 96 bp and 76 bp, whereas the affected individuals (A) produce an additional third fragment of 67 bp generated from the RsaI site created by the A → T change at codon 220.

Discussion

[0137] The isolation and characterization of the NF2 gene, encoding the *merlin* protein, creates new possibilities for accurate predictive testing in NF2. Until the NF2 gene was mapped to chromosome 22, no genetic testing was possible. Once the gene was mapped, it became possible to predict NF2 carrier status using linked DNA markers (Narod, S.A. *et al., Am. J. Hum. Genet. 51*:486-496 (1992)). Unfortunately, testing by linkage is limited by the availability of multiple family members for comparison, by lack of complete informativeness of linked markers, and by the diminished accuracy inherent in using a marker not located precisely within the disease gene. The precise identity of the NF2 gene discovered by the inventors now makes it possible to overcome these diagnostic hurdles by defining the exact molecular lesion associated with the disorder in any given pedigree.

[0138] Because mutations in NF2 were expected in general to inactivate the gene, they could have fallen into several different categories. DNA rearrangements, such as deletions, inversions, insertions, or duplications could be expected. Mutations that eliminate expression of the entire transcript, interfere with exon splicing, or disrupt its stability could effectively inactivate the gene. Finally, DNA sequence alterations within the coding sequence that cause premature termination of translation, yielding a truncated protein, or that result in a change in an amino acid residue critical for normal function of the *merlin* protein were also likely.

[0139] In this application, as shown in the exemplary material, the SSCP technique was applied as a rapid means to locate and confirm the probable molecular basis of NF2 in a large and well studied kindred (Wertelecki, W. *et al., New Engl. J. Med. 319*:278-283 (1988)). The technique used strategically placed primer sets to amplify small regions of the NF2 gene directly from genomic DNA using PCR. The findings were highly specific; none of the unaffected 158 control samples produced an altered SSCP pattern. As would be predicted, all samples from affected members of the same kindred produced the same altered SSCP pattern, reflecting the identical nature of the underlying molecular defect.

[0140] The sequence alteration in this family resulted in the substitution of a tyrosine residue for the asparagine at position 220 of the *merlin* protein. This change affects one of the most conserved stretches in the *merlin* protein as shown in Figure 12. This particular asparagine residue, and all surrounding residues are present in all three closely

[illegible] suggests that this region plays a [illegible] in the function of the protein. The change from asparagine to tyrosine would be expected to significantly disrupt the structure of this domain. The drastic nature of the amino acid change, and the absence of the same DNA change from more than 300 independent chromosomes argue

that this alteration is the cause of NF2 in this pedigree.

Example 6

Analysis of* merlin *in Sporadic and Inherited Tumors

[0141] To facilitate the search for mutations in NF2 and related tumors, the exon-intron junctions of the *NF2* locus were sequenced, including the E16 additional exon that is alternatively spliced (Bianchi, A.B., *et al.*, *Nature Genet.*, in press, 1994; Haase, V.H., *et al.*, *Human Mol. Genet.*, in press, 1994). PCR assays were developed for all seventeen exons, and were used to scan the entire gene for mutations in sporadic and inherited vestibular schwannomas. The high proportion of tumors in which inactivating mutations were found indicates that the *NF2* gene plays a fundamental role in schwannoma tumorigenesis.

Materials and Methods

Tissue samples

[0142] Tumor specimens were obtained at the time of surgery and frozen for DNA analysis. Blood samples were also obtained at the time of surgery to serve as normal tissue controls. High molecular weight DNA was extracted from peripheral blood leukocytes and from frozen pulverized tumor tissue by SDS-proteinase K digestion followed by phenol and chloroform extraction (Seizinger, B.R., *et al.*, *Nature 322*:644-647 (1986)).

Design of primer pairs

[0143] Exonic primers were designed within the *NF2* coding sequence near the intron-exon borders as determined by the results of exon trapping (Trofatter, J.A., *et al.*, *Cell 72*:791-800 (1993)). For those regions not isolated by trapping, primers were synthesized at approximate 100 base pair intervals. Using these primers, an intronic sequence was obtained by directly sequencing cosmids containing the gene using a cycle sequencing kit (US Biochemical). Intronic primer pairs were then designed to amplify the splice donor and acceptor sites as well as the exon itself. In the case of E12, it was necessary to construct two overlapping primer sets to maintain a product length of less than 300 base pairs.

SSCP analysis

[0144] SSCP analysis was performed according to the procedure of Orita *et al.* (Orita, M., *et al.*, *Genomics 5*:874-879 (1989)) with minor modifications. Approximately 50 ng of genomic DNA was amplified using appropriate intronic primer pairs (Table 2). Each 10 µl reaction contained 70 µM each of dATP, dCTP, dGTP and dTTP, 4 pmoles of each primer, 0.5 units Taq polymerase, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, and 0.1 µl $\alpha[^{33}P]$dATP (Amersham, 10 mCi/ml). For E1, a $MgCl_2$ concentration of 0.5 mM was used. Amplification was carried out for 30 cycles as follows: 94°C for 1 min., 55-60°C for 1 min., and 72°C for 1 min., after an initial denaturation step at 94°C for 4 min. One µl of labeled amplified DNA was diluted into 9 µl of 0.1% SDS and 10 mM EDTA, and an equal volume of loading dye (95% formamide, 0.5M EDTA, 0.05% bromphenol blue and 0.05% xylene cyanol) was added. The samples were denatured for 2 min. at 90°C and separated on 6-8% polyacrylamide gels containing 8% glycerol for 16 hrs. at 6-8W. Gels were dried and exposed to Kodak X-OMAT film.

DNA sequencing

[0145] For DNA sequencing, PCR amplifications were performed in 50 µl volumes as described above for SSCP analysis except that 200 µM dNTPs were added and a radioactive nucleotide was omitted. The product was sequenced by one of two methods. In the first, the double-stranded product was used as a template to generate single-strands by priming multiple rounds of DNA synthesis with one of the oligonucleotides previously used in the double-strand reaction (Gibbs, A., *et al.*, *Proc. Natl. Acad. Sci. USA 86*:1919-1923 (1989)). Conditions for asymmetric PCR amplification were identical except that only one primer was added. The product was ethanol precipitated in the presence of ammonium acetate and resuspended in 10 H_2O for subsequent sequence analysis. The sequencing reactions were performed [illegible] using Sequenase (T7 DNA polymerase, US Biochemical) under conditions [illegible] precipitate [illegible] standard [illegible] sequencing gels [illegible] DNA polymerase and the Circumvent Thermal Cycle kit (New England BioLabs). Both strands of exons with SSCP mobility shifts were analyzed in all cases.

Loss of heterozygosity analysis

[0146] Genomic DNA was amplified using primer pairs for the polymorphic dinucleotide repeats at markers *D22S193* (Trofatter *et al.*, in preparation) or *D22S268* (Marineau, C., *et al., Hum. Mol. Genet. 2*:336 (1993)). The CA strand primer was 5' end-labeled with polynucleotide kinase and gamma-^{32}P ATP, and PCR was performed as described previously (Louis, D.N., *et al., Am. J. Pathol. 141*:777-782 (1992)). In some cases, loss of chromosome 22 alleles was determined by Southern blot analysis using probes at the following loci: *D22S22, D22S29. D22S28, D22S15, D22S1, CRYB2, D22S10* and *D22S9* (Rouleau, G.A., *et al., Genomics* 4:1-6 (1989)).

Results

Exon Structure of the NF2 Gene

[0147] The internal exons range in size from 45 base pairs to 218 base pairs, with an average of 111 base pairs. Of the fifteen internal exons, ten (E3, E5-7, E9-11, E14-16) were isolated using exon amplification, the technique described herein, which led to the isolation of the *NF2* locus from cloned genomic DNA (see also Trofatter, J.A., *et al., Cell 72*:791-800 (1993)). Table 1 shows the DNA sequences immediately surrounding the intron-exon junctions, which all match the consensus for splice acceptor and donor sites. Additional intron sequences on both 5' and 3' sides that were used to design primers for PCR amplification are described herein in the section "Description of the Preferred Embodiments" (Figures 15A-Q herein).

PCR Assays for the Seventeen NF2 Gene Exons

[0148] For each internal exon, primers were chosen in flanking intron sequences to develop an assay for PCR amplification of the exon directly from genomic DNA. For E12, two overlapping primer sets were chosen to yield products in a size range amenable to SSCP analysis. Because E12 is 218 base pairs, two sets of primers were employed, one set spanning the 5' intron-exon junction and the 5' region of the exon and the other overlapping set spanning the remainder of the exon and the 3' exon-intron junction. In this way amplified fragments of a suitable length for SSCP analysis (140 and 284 base pairs, respectively) were generated. All of the coding region from E1 was amplified using one primer in the 5' untranslated region (UTR) and one in intron 1. For E17, a primer from the final intron was paired with a primer in the 3'UTR to amplify all of the E17 coding sequence, (along with the first 100 base pairs of 3'UTR). All assays (except that for E1) were performed according to standardized amplification conditions. As discussed in the exemplary material, E1 was amplified using a $MgCl_2$ concentration of 0.5 mM rather than 1.5 mM (to optimize the yield of PCR product). The annealing temperature was varied for optimum results. Table 2 lists the primers, annealing temperature and product size for each exon assay. In some cases (E7 and E8), an initial set of primers was used, but subsequently was replaced with a second primer pair. In some cases, the original primer was too far removed from the exon for convenient DNA sequencing or too close to the exon to detect potential intronic mutations. In such cases, primer pairs were used. In these instances both sets of primer pairs are listed.

Scanning for Mutations by SSCP Analysis of Blood-tumor Pairs

[0149] The exon PCR assays listen in Table 2 were used to scan the entire *merlin* coding sequence for mutations in schwannomas. SSCP analysis was applied to DNA extracted from thirty eight primary tumor specimens, including eight vestibular schwannomas from NF2 patients (Table 3), twenty-seven sporadic vestibular schwannomas (Table 4) and three sporadic spinal schwannomas (Table 4, S9, S25 and S27). DNA extracted from a blood sample of the corresponding individual was used for comparison in each case. All tumor-blood pairs were assayed for all exons.

[0150] Representative results of the SSCP analyses for three of the exons. (E2, E10 and E14) are shown in Figures 13A to 13C. For each assay, a lane of PCR product was run without denaturation (ND) to identify fully reannealed, double-stranded DNA in the test lanes. The other bands in the test lanes represent various conformations- of the single-stranded DNAs in the product. For E2, the normal pattern is seen only in the blood DNA (B) of S29. S11 and S33 display mobility shifts that are detected in both blood and tumor DNA. This result indicates germline alterations. S29 displays a mobility shift only in the tumor DNA. This result indicates a somatic mutation. For E10 and E14, all blood DNAs display the normal pattern and all tumor DNAs display different mobility shifts. Both normal and altered PCR products were compared by direct sequence analysis to identify the precise base change(s) involved.

Mutations in schwannoma

[0151] The blood and tumor DNAs were genotyped using polymorphic DNA markers to detect a loss of heteroz-

gosity that could indicate deletion of one *NF2* allele (Tables 3 and 4). General testing had predicted that at least sixteen of the thirty-eight tumors had lost one *NF2* allele. Thus, the entire *NF2* coding sequence for 58-60 independent alleles was examined by SSCP. No obvious polymorphism affecting coding or non-coding sequences was observed. This result indicates a remarkable degree of homogeneity for this gene sequence in the population. In contrast, mutations in both NF2 and sporadic tumors were readily detected. DNA differences from normal have been confirmed in twenty-seven of these tumors. Seven germline and twenty-five somatic alterations have been identified by sequence analysis. The results are summarized in Tables 3 and 4.

[0152] Germline mutations, present in both blood and tumor DNA, were delineated in five of the eight patients with a confirmed diagnosis of NF2 (Table 3). These changes occurred in disparate locations and included 1) point mutations creating stop codons at residues 57 and 60 of E2 (S11 and S33) and 1396 of E13 (S4); 2) a 28 base pair deletion creating a frame shift and premature stop codon in E10 (S1) and an insertion of one base into a splice donor site in E12 (S32). Two other germline alterations were found in blood and tumor DNA from individuals without a confirmed NF2 diagnosis (Table 4). These included 1) a single base change in the intron upstream of E7 in a sporadic spinal schwannoma (S9) and 2) a substitution of Cys for Arg at residue 418 in a sporadic vestibular schwannoma (S44). Although the two intron changes and the apparent missense mutation could conceivably represent polymorphisms, they were not found by SSCP analysis of 150 independent DNA samples from normal individuals or individuals with other types of tumors.

[0153] Somatic mutations were observed in tumors from five of the eight patients with NF2. These included two patients (S1 and S4) in whom a germline mutation was also detected (Table 3). In thirty sporadic tumors, a total of twenty somatic mutations were found (Table 4). In three cases in which chromosome 22 heterozygosity was maintained (S18, S24, and S29), two distinct somatic alterations were found in each tumor. For example, tumor S29 displayed small deletions of one base pair and four base pairs in E2 and E8, respectively (Figures 14A and 14B). In one tumor (S35), a complex of two adjacent deletions was detected in a single allele. One deletion removed five codons in-frame. The other deletion (beginning three base pairs downstream) caused a frameshift.

[0154] The twenty-five somatic mutations from NF2 and sporadic tumors were found throughout the gene and were associated with E1, E2, E3, E4, E7, E8, E9, E10, E12, E14 and E15. By far the most frequent lesions detected (19/25) were small deletions of one to sixty-one base pairs, that had either an obvious or presumed effect on splicing or that produced frameshifts that led to truncated proteins of altered sequence. A single mutation involved a frameshift resulting from a single base insertion (S42). The remaining five somatic changes were point mutations that either altered splice donor (S22) or acceptor (S12, S37) sites, produced a stop codon at residue 212 (S24), or generated a Met for Val substitution at residue 219 (S1).

Discussion

[0155] The results of the studies herein defining specific mutations immediately allow improved prenatal, presymptomatic and unaffected diagnosis for family members at risk. Highly accurate prenatal testing is now possible by direct examination of fetal DNA for the presence of characteristic SSCP shifts, altered DNA sequences, or gain or loss of restriction sites. Similarly, presymptomatic testing using these approaches could eliminate the need for up to half of at risk family members to undergo expensive and time consuming clinical monitoring, reducing the considerable financial and psychological burdens on these individuals. For those that test positive for the specific changes, medical care should be improved by the clarification of their status earlier in the course of their disease, which in turn might allow earlier consideration of surgical intervention. With the delineation of a larger number of mutations, it now becomes possible to discover whether the NF2 gene contains mutational "hot spots" which would simplify scanning.

[0156] NF2 is a disorder consistent with a "two-hit" model of tumorigenesis, in which homozygous inactivation of a gene that normally suppresses tumor growth is the critical event in tumor formation (Knudson, A.G., *Proc. Natl. Acad. Sci. USA 68*:820-823 (1971)). The same types of tumors that are present as multiple independent growths in NF2 patients occur as sporadic, solitary cases in the general population (Martuza and Eldridge, *New Eng. J. Med. 318*:684-688 (1988); Mulvihill, J., *et al., Ann. Intern. Med. 113*:39-52 (1990)). The familial and sporadic tumors both display frequent loss of genetic material from chromosome 22, in a region to which the *NF2* gene defect has been mapped by linkage analysis (Kaiser-Kupfer, M.I., *et al., Arch. Ophthalmol. 107*:541-544 (1989); Rouleau, G.A., *et al., Nature 329*:246-248 (1987); Wertelecki, W., *et al., New Engl. J. Med. 319*:278-283 (1988); Rouleau, G.A., *et al., Am. J. Hum Genet. 46*:323-328 (1990); Narod, S.A., *et al., Am. J. Hum. Genet. 51* 486-496 (1992); Seizinger, B.R., *et al., Nature 322*:644-647 (1986); Seizinger, B.R., *et al., Science 236*:317-319 (1987); Seizinger, B.R., *et al., Proc. Natl. Acad. Sci.* [illegible] 15:55-62 (1990); Bijlsma, E.K., *et al., Genes*

[illegible] 478-485 (1992)). Thus, it is presumed that the [illegible] locus encodes a tumor suppressor and that inactivation of both alleles by loss or mutation in specific cells results in unregulated proliferation. However, only specific cell types are

affected in this way as the vast majority of tumors seen in NF2 are schwannomas, and particularly vestibular schwannomas and meningiomas.

[0157] The results herein implicate *merlin* as a tumor suppressor. Whereas germline mutations are present in both blood and tumor DNA from NF2 patients, somatic mutation of *merlin* is a frequent event in schwannomas. In several cases, two inactivating mutations were detected in the same tumor. In many others, a single mutant allele remained following the loss of the second copy of the locus. The alterations occured throughout the *NF2* gene. Most exons displayed at least one mutation.

[0158] One alteration, conversion of the Arg codon at position 57 to a stop codon, has been seen twice before in independent NF2 patients (Rouleau, G.A., *et al., Nature 363*:515-521 (1993)), suggesting that this site containing a cCpG dinucleotide may be particularly prone to C → T transitions. The presence of this change in the blood DNA of S11 combined with this absence from either parent demonstrate that this is a case of a new mutation to NF2.

[0159] Two new missense mutations were identified, Val219Met and Arg418Cys, which may target these residues as particularly important in *merlin's* tumor suppressor function. The Val at position 219, one residue away from the previously reported Asn220Tyr mutation, is located within the protein 4.1 domain that is characteristic of this family and is conserved in human moesin, radixin, and protein 4.1, but changed to Ile in human ezrin. The Arg at position 418 is located in the long α-helical domain that comprises most of the C-terminal half of *merlin* and its relatives but is not strictly conserved in the other human members of this protein family.

[0160] A surprising number of somatic mutations involve changes in intron sequences. These may occur at a distance from the exon-intron junction. The absence of these alterations in blood-derived DNA of the same individuals, and the failure to detect the same change in any other individuals, indicate that these mutations are *de novo* events associated with tumor formation.

[0161] In addition to expanding the number and variety of germline *NF2* mutations described, the examples herein suggest that germline alterations occur in patients not yet diagnosed clinically with NF2. One patient, with a single spinal schwannoma, displayed a single base alteration in an intron sequence. Another with a single vestibular schwannoma, displayed the Arg418Cys change described above. Careful clinical follow-up is indicated for these patients. It is possible that they represent a class of individuals with mutations that only mildly affect normal *merlin* function, and consequently do not produce the full NF2 phenotype.

[0162] The development of reliable PCR assays for each exon of the *NF2* gene should facilitate greatly the cataloging of mutations in NF2 patients and their tumors by genomic scanning. It can be expected that a detailed mutational analysis of the *NF2* gene, identifying sites particularly prone to alteration, pinpointing amino acid residues crucial for normal function, and providing a basis for relating specific alterations with variations in phenotype will result. Perhaps most important, however, the ability to rapidly scan the *NF2* gene for mutations will accelerate the assessment of a role for *merlin* in other tumor types.

Table 1

Intron-Exon Boundaries of the *NF2* Gene

Exon	Splice Acceptor	Start (bp)[1]	Exon Start	Exon Length	Exon End	End (bp)	Splice Donor
1					GAG	114	GTAACCGGCC
2	GTTATTGCAG	115	ATG	126	AAG	240	GTTGGGCTAG
3	AATTCTGCAG	241	GTA	123	CAG	363	GTACATCAGT
4	CTCCTTTCAG	364	GTA	84	AAG	447	GTAGGCTCAA
5	TTCTTTCCAG	448	TAT	69	AGG	516	GTAAGAGATT
6	TTTTTGGTAG	517	GTA	83	CAG	599	GTGAGGCCCA
7	CTCCCCACAG	600	GGA	76	CGG	675	GTGTGTTGAA
8	GGATCCACAG	676	AAT	135	GAG	810	GTAGGACATG
9	ATTCTTCCAG	811	TTT	75	CTG	885	GTAAGTTGAG
10	GTGGCCACAG	886	ATT	114	CAG	999	GTGAGCACAA
11	CCCCTCGCAG	1000	ATG	123	CTG	1122	GTGATTTCTG
12	TGCCCTCCAG	1123	ATG	218	GAG	1340	GTGAGGGGGC
13	TTGCTTGCAG	1341	GGC	106	CCG	1446	GTGAGCCTGG
14	TCATTAACAG	1447	CCC	128	AAA	1574	GTATGTAGCC
15	TTGCCGGCAG	1575	AGT	163	AAG	1737	GTACCCAGGG
16	GCTGGTTTAG	1738	CCT	45	AAA	1782	GTAGGTTGTT
17	TTTCTTACAG	1783	CTC				

[1] Base pair numbering is based on #1 being the A of the initiator ATG

Table 2

Primers for Exon PCR Assays

Exon	Product	Temp (°C)	Primer #1 (5'→3')	Primer #2 (5'→3')
1	246	58	GCTAAAGAGCTCAGAGTGCAG	GAGAAGCTCTGGAGCTTCCAC
2	182	60	TGTCCTTCCCCATTGCTTTG	CACTTTCATCGAGTTCTAGCC
	248	58	AGTCCAGAGAAAAGGTTTTAATAAATGAT	TGCAAAGCTCACGTCAGCC
3	272	60	GCTTCTTTGGGGTAGCACA	GGTCAACTCTGAGGCCAACT
4	188	59	CCTCACTTCCCTCACAGAG	CCCATGACCCAAATTAACGC
5	148	60	GCTCTCCCTTTCTTCTTGC	TGCTTCAAGTGCTTTGGTTAGC
	171	58	TGGCAGTTATCTTTAGAATCTC	TTAGACCACATATCTGCTATG
6	161	60	CATGTGTAGGTTTTTTATTTTGC	GCCCATAAAGGAATGTAAACC
7	173	60	CCACCTCACTTAGCTCCAATG	GTCACTCAGTCTCT TCTAC
	170	60	GAATGCTTCATTTCGCGCTC	GAGGTTTCAACACACCCGGA
8	247	60	GAAGGTTGAATAAAATTTCAGAGCCTC	GACAGGGAAAGATCTGCTGGACC
	232	60	CTGTTCTTATTGGATCCACAG	AACAACCACACCCTCAAAGC
9	300	58	GACTTGGTGCTCCTAATTCCC	CCATTATCAGTAATGAAAACCAGG
10	260	59	TGCTACCTGCAAGAGCTCAA	CTGACCACACAG TGACATC
11	268	60	TCTTTGGCCCTTGTGGCAC	CAGGAGACCAAGCTCCAGAA
12A	340	60	TTCAGCTAAGAGCACTGTGC	CGCTGCATTTCCTGCTCAG
12B	204	58	GCTGAAAAGGCCCAGATCA	CTTGAGGACAACTGCTGTAG
13	228	60	GGTGTCTTTTCCTGCTACCT	GGGAGGAAAGAGAACATCAC
14	251	60	TGTGCCATTGCCTCTCTG	AGGGCACAGGGGGCTACA
15	317	58	TGGCAAGTAGAGACGTGA	TACAAGAAAGAGACCCTCCC
	248	58	TCTCCCCAAGCCCTGATGC	TGGTCCTGATCAGCAAAATAC
16	138	60	GGCATTGTTGATATCACAGGG	GGCAGCACCATCACCACATA
17	177	60	CTCTCAGCTTCTTCTCTGCT	CCAGCCAGCTCCTATGGATG

	All exons were examined by single PCR assays except exon 12, for which overlapping assays (12A and 12B) were required. For exons 2, 3, 7, 8 and 15, two different PCR assays were developed.
*	Annealing temperature for PCR reaction

Table 3

NF2 Gene Mutations in Schwannomas from NF2 Patients

Tumor	Exon	DNA Sequence Alteration[1]	Codon Change[2]	Consequence	Origin[3]	Alleles[4]
S1	E7	655 G > A	Val219Met	Missense	S	2
S1	E10	907/6 to 931/3 del 28bp	Gly302fs > 322X	Frameshift	G	
S1	E3	353 to 361+19 del 30bp		Splice donor site	S	2
S4	E11	1396 C > T	Arg466X	Nonsense	G	
S10	E9	844 del 1 bp (G)	Val282fs > 296X	Frameshift	S	2
S11	E2	169 C > T	Arg57X	Nonsense	G	2
S12	E12	1410+2 (not T) bp (C)		Splice donor ?	G	2
S13	E2	179 G > A	Trp60X	Nonsense	G	1
S34	E14	1451 to 1452 del 2 bp (TG)	Met184fs > 494X	Frameshift	S	NI
S36	E7	600-28 to -5 del 24bp		Splice acceptor?	S	2

[1] Numbering of bases showing alteration is given relative to the cDNA sequence with the initiator ATG beginning at base 1. All coding sequence bases are given in upper case. When the alteration affects intronic sequence, it is presented in lower case and numbered as "-" (5' intron) or "+" (3' intron) the requisite number of bases from the first or last base of the exon, respectively. For deletions, the span of deleted bases (numbered as above) is given, followed by the deletion size ("del"). For deletions of less than 5 bp, the deleted base are also named. Where the start position of the deletion is uncertain, the alternative ranges of bases deleted are shown. Insertion is indicated by "ins" followed by the number of bases inserted, and their identity

[2] Original amino acid and position of the residues in the protein (numbered from the initiator Met as 1) is followed by new amino acid for missense mutation, X for nonsense mutation, or fs for frameshift, followed by the position of the next in-frame stop codon.

[3] S = somatic mutation, G = germline mutation

[4] Number of *NF2* alleles in tumor predicted by heterozygosity testing with Chr 22 DNA markers. NI = not informative

Table 4
NF2 Gene Mutations in Sporadic Schwannomas[1]

Tumor	Exon	DNA Sequence Alteration	Codon Change	Consequence	Origin	Alleles
S2	E3	241-22 to -13 del 10bp		Splice acceptor?	S	1
S3						NI
S5						1
S6						2
S9	E7	600-32 t->a		Acceptor branch site?	C	2
S12	E7	600-1 g->a		Splice acceptor site	S	1
S13						2
S14						1
S15	E15	1634/6 to 1694/6 del 61bp	Ile544fs -> 566X	Frameshift	S	1
S16	E4	339 del T (C)	Gln147fs -> 174X	Frameshift	S	2
S17						2
S18	E8	676-10 to 726 del 61bp		Splice acceptor site	S	2
	E12	1266 to 1267 del 2 (GA)	Glu422fs -> 442X	Frameshift	S	
S19	E15	1575 46/-27 to 1591/2 del 34bp		Splice acceptor site	S	1
S22	E7	675+1 g->t		Splice donor site	S	1
S23						1
S24	E7	634 C->T	Gln212X	Nonsense	S	2
	E10	905 to 912 del 8bp	Gly302fs -> 331X	Frameshift	S	
S25	E10	992 to 999 +1 del 9bp		Splice donor site	S	1
S26						1

Table 4
NF2 Gene Mutations in Sporadic Schwannomas[1]

Tumor	Exon	DNA Sequence Alteration	Codon Change	Consequence	Origin	Alleles
S27						2
S29	E2	134 del 1 (A)	Asp45fs > 123X	Frameshift	S	2
	E8	729 to 732 del 4 (TTAT)	Ile243fs > 251X	Frameshift	S	
S30	E4	447 or 447+1 del 1 (G or g)	Lys149fs > 174X	Frameshift or splice donor site	S	1
S31						1
S35	E1	65/70 to 79/84 del 15bp 88 to 109 del 22bp	del 5 aa Asp30fs > 40X	Frameshift	S	1
S37	E4	364-2 A->G		Splice acceptor site	S	2
S38	E10	933 del 1 (G)	Arg311fs > 322X	Frameshift	S	2
S39	E12	1223 to 1227 del 5bp	Glu408fs > 442X	Frameshift	S	1
S40						1
S42	E14	1517/20 ins 1 (T)	Phe507fs > 513X	Frameshift	S	2
S43	E14	1571/4 del 1 (A)	Lys525fs > 550X	Frameshift	S	2
S44	E12	1252 C->T	Arg418Cys	Missense	G	2

[1] Explanation of all symbols can be found in Table 1.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE GENERAL HOSPITAL CORPORATION
Fruit Street
Boston, Massachusetts 02114
United States of America

INVENTORS: Trofatter, James A.
MacCollin, Mia M.
Gusella, James F.

(ii) TITLE OF INVENTION: Tumor Suppressor Gene Merlin and Uses Thereof

(iii) NUMBER OF SEQUENCES: 118

(iv) AGENTS ADDRESS:
(A) ADDRESSEE: Kilburn & Strode
(B) STREET: 30 John Street
(C) CITY: LONDON
(D) COUNTRY: England
(E) POSTAL CODE: WC1N 2DD

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) FILING DATE: 25th February 1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US (to be assigned)
(B) FILING DATE: 22-DEC-1993

(viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/108,808
(B) FILING DATE: 19-AUG-1993

(viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/022,034
(B) FILING DATE: 25-FEB-1993

(viii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/026,063
(B) FILING DATE: 04-MAR-1993

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGATTGTTC ATTCCAAGTG G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACCCTGAGGA ATCCACTACC 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGCACACACA TCCTTTTCAC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAGAGAGACT GCTGTCTCAA AAA 23

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGGAGGCTGA ACGCACGAG 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGGTATTGTG CTTGCTGCTG 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTTCAACCTG ATTGGTGACA G 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGGTATTGTG CTTGCTGGTG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTACTGGA TCATGATGTT TC 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTTGGAAGCA ATTCCTCTTG G 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCAGCCAGCT CCCTATGGAT G 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGCTGAAATG GAATATCTGA AG 22

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCCTTCTCCT CCCTGGCCTG 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GATGGAGTTC AATTGCGAGA TG 22

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2257 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 220..2004

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
ACGGCAGCCG TCAGGGACCG TCCCCCAACT CCCCTTTCCG CTCAGGCAGG GTCCTCGCGG      60

CCCATGCTGG CCGCTGGGGA CCCGCGCAGC CCAGACCGTT CCCGGGCCGG CCAGCCGGCA     120

CCATGGTGGC CCTGAGGCCT GTGCAGCAAC TCCAGGGGGG CTAAAGGGCT CAGAGTGCAG     180

GCCGTGGGGC GCGAGGGTCC CGGGCCTGAG CCCCGCGCC ATG GCC GGG GCC ATC         234
                                              Met Ala Gly Ala Ile
                                               1               5

GCT TCC CGC ATG AGC TTC AGC TCT CTC AAG AGG AAG CAA CCC AAG ACG        282
Ala Ser Arg Met Ser Phe Ser Ser Leu Lys Arg Lys Gln Pro Lys Thr
            10                  15                  20
```

```
TTC ACC GTG AGG ATC GTC ACC ATG GAC GCC GAG ATG GAG TTC AAT TGC      330
Phe Thr Val Arg Ile Val Thr Met Asp Ala Glu Met Glu Phe Asn Cys
                25                  30                  35

GAG ATG AAG TGG AAA GGG AAG GAC CTC TTT GAT TTG GTG TGC CGG ACT      378
Glu Met Lys Trp Lys Gly Lys Asp Leu Phe Asp Leu Val Cys Arg Thr
            40                  45                  50

CTG GGG CTC CGA GAA ACC TGG TTC TTT GGA CTG CAG TAC ACA ATC AAG      426
Leu Gly Leu Arg Glu Thr Trp Phe Phe Gly Leu Gln Tyr Thr Ile Lys
        55                  60                  65

GAC ACA GTG GCC TGG CTC AAA ATG GAC AAG AAG GTA CTG GAT CAT GAT      474
Asp Thr Val Ala Trp Leu Lys Met Asp Lys Lys Val Leu Asp His Asp
    70                  75                  80                  85

GTT TCA AAG GAA GAA CCA GTC ACC TTT CAC TTC TTG GCC AAA TTT TAT      522
Val Ser Lys Glu Glu Pro Val Thr Phe His Phe Leu Ala Lys Phe Tyr
                90                  95                  100

CCT GAG AAT GCT GAA GAG GAG CTG GTT CAG GAG ATC ACA CAA CAT TTA      570
Pro Glu Asn Ala Glu Glu Glu Leu Val Gln Glu Ile Thr Gln His Leu
            105                 110                 115

TTC TTC TTA CAG GTA AAG AAG CAG ATT TTA GAT GAA AAG ATC TAC TGC      618
Phe Phe Leu Gln Val Lys Lys Gln Ile Leu Asp Glu Lys Ile Tyr Cys
        120                 125                 130

CCT CCT GAG GCT TCT GTG CTC CTG GCT TCT TAC GCC GTC CAG GCC AAG      666
Pro Pro Glu Ala Ser Val Leu Leu Ala Ser Tyr Ala Val Gln Ala Lys
    135                 140                 145

TAT GGT GAC TAC GAC CCC AGT GTT CAC AAG CGG GGA TTT CTG GCC CAA      714
Tyr Gly Asp Tyr Asp Pro Ser Val His Lys Arg Gly Phe Leu Ala Gln
150                 155                 160                 165

GAG GAA TTG CTT CCA AAA AGG GTA ATA AAT CTG TAT CAG ATG ACT CCG      762
Glu Glu Leu Leu Pro Lys Arg Val Ile Asn Leu Tyr Gln Met Thr Pro
                170                 175                 180

GAA ATG TGG GAG GAG AGA ATT ACT GCT TGG TAC GCA GAG CAC CGA GGC      810
Glu Met Trp Glu Glu Arg Ile Thr Ala Trp Tyr Ala Glu His Arg Gly
            185                 190                 195

CGA GCC AGG GAT GAA GCT GAA ATG GAA TAT CTG AAG ATA GCT CAG GAC      858
Arg Ala Arg Asp Glu Ala Glu Met Glu Tyr Leu Lys Ile Ala Gln Asp
        200                 205                 210

CTG GAG ATG TAC GGT GTG AAC TAC TTT GCA ATC CGG AAT AAA AAG GGC      906
Leu Glu Met Tyr Gly Val Asn Tyr Phe Ala Ile Arg Asn Lys Lys Gly
    215                 220                 225

ACA GAG CTG CTG CTT GGA GTG GAT GCC CTG GGG CTT CAC ATT TAT GAC      954
Thr Glu Leu Leu Leu Gly Val Asp Ala Leu Gly Leu His Ile Tyr Asp
230                 235                 240                 245

CCT GAG AAC AGA CTG ACC CCC AAG ATC TCC TTC CCG TGG AAT GAA ATC     1002
Pro Glu Asn Arg Leu Thr Pro Lys Ile Ser Phe Pro Trp Asn Glu Ile
                250                 255                 260

CGA AAC ATC TCG TAC AGT GAC AAG GAG TTT ACT ATT AAA CCA CTG GAT     1050
Arg Asn Ile Ser Tyr Ser Asp Lys Glu Phe Thr Ile Lys Pro Leu Asp
            265                 270                 275

AAG AAA ATT GAT GTC TTC AAG TTT AAC TCC TCA AAG CTT CGT GTT AAT     1098
Lys Lys Ile Asp Val Phe Lys Phe Asn Ser Ser Lys Leu Arg Val Asn
        280                 285                 290

AAG CTG ATT CTC CAG CTA TGT ATC GGG AAC GAT GAC CTA TTT ATG AGG     1146
Lys Leu Ile Leu Gln Leu Cys Ile Gly Asn Asp Asp Leu Phe Met Arg
    295                 300                 305
```

```
AGA AGG AAA GCC GAT TCT TTG GAA GTT CAG CAG ATG AAA GCC CAG GCC      1194
Arg Arg Lys Ala Asp Ser Leu Glu Val Gln Gln Met Lys Ala Gln Ala
310                 315                 320                 325

AGG GAG GAG AAG GCT AGA AAG CAG ATG GAG CGG CAG CGC CTC GCT CGA      1242
Arg Glu Glu Lys Ala Arg Lys Gln Met Glu Arg Gln Arg Leu Ala Arg
                330                 335                 340

GAG AAG CAG ATG AGG GAG GAG GCT GAA CGC ACG AGG GAT GAG TTG GAG      1290
Glu Lys Gln Met Arg Glu Glu Ala Glu Arg Thr Arg Asp Glu Leu Glu
            345                 350                 355

AGG AGG CTG CTG CAG ATG AAA GAA GAA GCA ACA ATG GCC AAC GAA GCA      1338
Arg Arg Leu Leu Gln Met Lys Glu Glu Ala Thr Met Ala Asn Glu Ala
        360                 365                 370

CTG ATG CGG TCT GAG GAG ACA GCT GAC CTG TTG GCT GAA AAG GCC CAG      1386
Leu Met Arg Ser Glu Glu Thr Ala Asp Leu Leu Ala Glu Lys Ala Gln
    375                 380                 385

ATC ACC GAG GAG GAG GCA AAA CTT CTG GCC CAG AAG GCC GCA GAG GCT      1434
Ile Thr Glu Glu Glu Ala Lys Leu Leu Ala Gln Lys Ala Ala Glu Ala
390                 395                 400                 405

GAG CAG GAA ATG CAG CGC ATC AAG GCC ACA GCG ATT CGC ACG GAG GAG      1482
Glu Gln Glu Met Gln Arg Ile Lys Ala Thr Ala Ile Arg Thr Glu Glu
                410                 415                 420

GAG AAG CGC CTG ATG GAG CAG AAG GTG CTG GAA GCC GAG GTG CTG GCA      1530
Glu Lys Arg Leu Met Glu Gln Lys Val Leu Glu Ala Glu Val Leu Ala
            425                 430                 435

CTG AAG ATG GCT GAG GAG TCA GAG AGG AGG GCC AAA GAG GCA GAT CAG      1578
Leu Lys Met Ala Glu Glu Ser Glu Arg Arg Ala Lys Glu Ala Asp Gln
        440                 445                 450

CTG AAG CAG GAC CTG CAG GAA GCA CGC GAG GCG GAG CGA AGA GCC AAG      1626
Leu Lys Gln Asp Leu Gln Glu Ala Arg Glu Ala Glu Arg Arg Ala Lys
    455                 460                 465

CAG AAG CTC CTG GAG ATT GCC ACC AAG CCC ACG TAC CCG CCC ATG AAC      1674
Gln Lys Leu Leu Glu Ile Ala Thr Lys Pro Thr Tyr Pro Pro Met Asn
470                 475                 480                 485

CCA ATT CCA GCA CCG TTG CCT CCT GAC ATA CCA AGC TTC AAC CTC ATT      1722
Pro Ile Pro Ala Pro Leu Pro Pro Asp Ile Pro Ser Phe Asn Leu Ile
                490                 495                 500

GGT GAC AGC CTG TCT TTC GAC TTC AAA GAT ACT GAC ATG AAG CGG CTT      1770
Gly Asp Ser Leu Ser Phe Asp Phe Lys Asp Thr Asp Met Lys Arg Leu
            505                 510                 515

TCC ATG GAG ATA GAG AAA GAA AAA GTG GAA TAC ATG GAA AAG AGC AAG      1818
Ser Met Glu Ile Glu Lys Glu Lys Val Glu Tyr Met Glu Lys Ser Lys
        520                 525                 530

CAT CTG CAG GAG CAG CTC AAT GAA CTC AAG ACA GAA ATC GAG GCC TTG      1866
His Leu Gln Glu Gln Leu Asn Glu Leu Lys Thr Glu Ile Glu Ala Leu
    535                 540                 545

AAA CTG AAA GAG AGG GAG ACA GCT CTG GAT ATT CTG CAC AAT GAG AAC      1914
Lys Leu Lys Glu Arg Glu Thr Ala Leu Asp Ile Leu His Asn Glu Asn
550                 555                 560                 565

TCC GAC AGG GGT GGC AGC AGC AAG CAC AAT ACC ATT AAA AAG CTC ACC      1962
Ser Asp Arg Gly Gly Ser Ser Lys His Asn Thr Ile Lys Lys Leu Thr
                570                 575                 580
```

```
TTG CAG AGC GCC AAG TCC CGA GTG GCC TTC TTT GAA GAG CTC                2004
Leu Gln Ser Ala Lys Ser Arg Val Ala Phe Phe Glu Glu Leu
            585                 590                 595

TAGCAGGTGA CCCAGCCACC CCAGGACCTG CCACTTCTCC TGCTACCGGG ACCGCGGGAT     2064

GGACCAGATA TCAAGAGAGC CATCCATAGG GAGCTGGCTG GGGGTTTCCG TGGGAGCTCC     2124

AGAAGTTTCC CCAGCTGAGT CAAGAGCCCA GCCCCTCTTA TGTGCAATTG CCTTGAACTA     2184

CGACCCTGTA GAGATTTCTC TCATGGCGTT CTAGTTCTCT GACCTGAGTC TTTGTTTTAA     2244

GAAGTATTTG TCT                                                        2257
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 595 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Met Ala Gly Ala Ile Ala Ser Arg Met Ser Phe Ser Ser Leu Lys Arg
 1               5                  10                  15

Lys Gln Pro Lys Thr Phe Thr Val Arg Ile Val Thr Met Asp Ala Glu
            20                  25                  30

Met Glu Phe Asn Cys Glu Met Lys Trp Lys Gly Lys Asp Leu Phe Asp
        35                  40                  45

Leu Val Cys Arg Thr Leu Gly Leu Arg Glu Thr Trp Phe Phe Gly Leu
    50                  55                  60

Gln Tyr Thr Ile Lys Asp Thr Val Ala Trp Leu Lys Met Asp Lys Lys
65                  70                  75                  80

Val Leu Asp His Asp Val Ser Lys Glu Glu Pro Val Thr Phe His Phe
                85                  90                  95

Leu Ala Lys Phe Tyr Pro Glu Asn Ala Glu Glu Glu Leu Val Gln Glu
            100                 105                 110

Ile Thr Gln His Leu Phe Phe Leu Gln Val Lys Lys Gln Ile Leu Asp
        115                 120                 125

Glu Lys Ile Tyr Cys Pro Pro Glu Ala Ser Val Leu Leu Ala Ser Tyr
    130                 135                 140

Ala Val Gln Ala Lys Tyr Gly Asp Tyr Asp Pro Ser Val His Lys Arg
145                 150                 155                 160

Gly Phe Leu Ala Gln Glu Glu Leu Leu Pro Lys Arg Val Ile Asn Leu
                165                 170                 175

Tyr Gln Met Thr Pro Glu Met Trp Glu Glu Arg Ile Thr Ala Trp Tyr
            180                 185                 190

Ala Glu His Arg Gly Arg Ala Arg Asp Glu Ala Glu Met Glu Tyr Leu
        195                 200                 205

Lys Ile Ala Gln Asp Leu Glu Met Tyr Gly Val Asn Tyr Phe Ala Ile
    210                 215                 220

Arg Asn Lys Lys Gly Thr Glu Leu Leu Leu Gly Val Asp Ala Leu Gly
225                 230                 235                 240

Leu His Ile Tyr Asp Pro Glu Asn Arg Leu Thr Pro Lys Ile Ser Phe
                245                 250                 255
```

```
Pro Trp Asn Glu Ile Arg Asn Ile Ser Tyr Ser Asp Lys Glu Phe Thr
            260                 265                 270

Ile Lys Pro Leu Asp Lys Lys Ile Asp Val Phe Lys Phe Asn Ser Ser
        275                 280                 285

Lys Leu Arg Val Asn Lys Leu Ile Leu Gln Leu Cys Ile Gly Asn His
    290                 295                 300

Asp Leu Phe Met Arg Arg Arg Lys Ala Asp Ser Leu Glu Val Gln Gln
305                 310                 315                 320

Met Lys Ala Gln Ala Arg Glu Glu Lys Ala Arg Lys Gln Met Glu Arg
                325                 330                 335

Gln Arg Leu Ala Arg Glu Lys Gln Met Arg Glu Glu Ala Glu Arg Thr
            340                 345                 350

Arg Asp Glu Leu Glu Arg Arg Leu Leu Gln Met Lys Glu Glu Ala Thr
        355                 360                 365

Met Ala Asn Glu Ala Leu Met Arg Ser Glu Glu Thr Ala Asp Leu Leu
    370                 375                 380

Ala Glu Lys Ala Gln Ile Thr Glu Glu Glu Ala Lys Leu Leu Ala Gln
385                 390                 395                 400

Lys Ala Ala Glu Ala Glu Gln Glu Met Gln Arg Ile Lys Ala Thr Ala
                405                 410                 415

Ile Arg Thr Glu Glu Glu Lys Arg Leu Met Glu Gln Lys Val Leu Glu
            420                 425                 430

Ala Glu Val Leu Ala Leu Lys Met Ala Glu Glu Ser Glu Arg Arg Ala
        435                 440                 445

Lys Glu Ala Asp Gln Leu Lys Gln Asp Leu Gln Glu Ala Arg Glu Ala
    450                 455                 460

Glu Arg Arg Ala Lys Gln Lys Leu Leu Glu Ile Ala Thr Lys Pro Thr
465                 470                 475                 480

Tyr Pro Pro Met Asn Pro Ile Pro Ala Pro Leu Pro Pro Asp Ile Pro
                485                 490                 495

Ser Phe Asn Leu Ile Gly Asp Ser Leu Ser Phe Asp Phe Lys Asp Thr
            500                 505                 510

Asp Met Lys Arg Leu Ser Met Glu Ile Glu Lys Glu Lys Val Glu Tyr
        515                 520                 525

Met Glu Lys Ser Lys His Leu Gln Glu Gln Leu Asn Glu Leu Lys Thr
    530                 535                 540

Glu Ile Glu Ala Leu Lys Leu Lys Glu Arg Glu Thr Ala Leu Asp Ile
545                 550                 555                 560

Leu His Asn Glu Asn Ser Asp Arg Gly Gly Ser Ser Lys His Asn Thr
                565                 570                 575

Ile Lys Lys Leu Thr Leu Gln Ser Ala Lys Ser Arg Val Ala Phe Phe
            580                 585                 590

Glu Glu Leu
        595
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCATCTCACT TAGCTCCAAT G 21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCACTCAGT CTCTGTCTAC 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTAACCGGCC 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTTATTGCAG 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTGGGCTAG 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATTCTGCAG 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTACATCAGT 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCCTTTCAG 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTAGGCTCAA 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTCTTTCCAG 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTAAGAGATT 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION. SEQ ID NO:28:

TTTTTGGTAG 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION· SEQ ID NO:29:

GTGAGGCCCA 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CTCCCCACAG 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION. SEQ ID NO:31:

GTGTGTTGAA 10

(2) INFORMATION FOR SEQ ID NO 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGATCCACAG 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTAGGACATG 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ATTCTTCCAG 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTAAGTTGAG 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTGGCCACAG 10

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTGAGCACAA 10

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CCCCTCGCAG 10

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTGATTTCTG 10

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGCCCTCCAG 10

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GTGAGGGGGC 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TTCCTTGCAG 10

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GTGAGCCTGG 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TCATTAACAG 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTATGTAGCC 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTGCCGGCAG 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GTACCCAGGG 10

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCTGGTTTAG 10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GTAGGTTGTT 10

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TTTCTTACAG 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GCTAAAGGGC TCAGAGTGCA G 21

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAGAACCTCT CGAGCTTCCA C 21

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TGTCCTTCCC CATTGGTTTG 20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CAGTTTCATC GAGTTCTAGC C 21

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

AGTGCAGAGA AAAGGTTTTA TTAATGAT 28

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TGGAAAGCTC ACGTCAGCC 19

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GCTTCTTTGA GGGTAGCACA 20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGTCAACTCT GAGGCCAACT 20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CCTCACTTCC CCTCACAGAG 20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCCATGACCC AAATTAACGC 20

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GCTCTCCCTT TCTTCTTTCC 20

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TCCTTCAAGT CCTTTGGTTA GC 22

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TGGCAGTTAT GTTTAGAATC TC 22

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TTAGACCACA TATCTGCTAT G 21

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CATGTGTAGG TTTTTTATTT TGC 23

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GCCCATAAAG GAATGTAAAC C 21

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CCATCTCACT TAGCTCCAAT G 21

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

CTCACTCAGT CTCTGTCTAC 20

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GAATGCTTGA TTTGGTGCCC 20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GAGGTTTCAA CACACCCGGA 20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GAAGGTTGAA TAAAATTTTG AGCCTC 26

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GACAGGGAAA GATCTGCTGG ACC 23

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CTGTTCTTAT TGGATCCACA G 21

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

AACAACCACA CCCTCAAAGC 20

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GACTTGGTGC TCCTAATTCC C 21

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CCATTATCAG TAATGAAAAC CAGG 24

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TGCTACCTGC AAGAGCTCAA 20

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CTGACCACAC AGTGACATC 19

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

TCTTTGGCCC TTGTGGCAC 19

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

CAGGAGACCA AGCTCCAGAA 20

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TTCAGCTAAG AGCACTGTGC 20

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CGCTGCATTT CCTGCTCAG 19

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCTGAAAAGG CCCAGATCA 19

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CTTGAGGACA ACTGCTGTAG 20

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GGTGTCTTTT CCTGCTACCT 20

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGGAGGAAAG AGAACATCAC 20

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TGTGCCATTG CCTCTGTG 18

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

AGGGCACAGG GGGCTACA 18

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TGGCCAAGTA GAGACGTGA 19

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

TACAAGAAAG AGACCCTGGG 20

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

TCTGCCCAAG CCCTGATGC 19

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

TGGTCCTGAT CAGCAAAATA C 21

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GGCATTGTTG ATATCACAGG G 21

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94

GGCAGCACCA TCACCACATA 20

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CTCTCAGCTT CTTCTCTGCT 20

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CCAGCCAGCT CCTATGGATG 20

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

TACGGTGTGW ACTACTTTGC A 21

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TAC GGT GTG AAC TAC TTT GCA 21
Tyr Gly Val Asn Tyr Phe Ala
1 5

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Tyr Gly Val Asn Tyr Phe Ala
1 5

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GGGAAGGACC TCTTT 15

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

CACATTTATG ACCC 14

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

AGTGCAGACA AAAGGTTTTA TTAATGATTT TTGCTCACAG TGTCCTTCCC CATTGGTTTG 60

TTATTGCAGA TGAAGTGGAA AGGGAAGGAC CTCTTTGATT TGGTGTGCCG GACTCTGGGG 120

CTCCGAGAAA CCTGGTTCTT TGGACTGCAG TACACAATCA AGGACACAGT GGCCTGGCTC 180

AAAATGGACA AGAAGGTTGG GCTAGAACTC GATGAAACTG GTGGGGCTGA CGTGAGCTTT 240

CCA 243

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GCTTCTTTGA GGGTAGCACA GGAGGAAGTG CCAATATANN GTGTGTTTGT CTTTTGCTCT 60

GCAATTCTGC AGGTACTGGA TCATGATGTT TCAAAGGAAG AACCAGTCAC CTTTCACTTC 120

TTGGCCAAAT TTTATCCTGA GAATGCTGAA GAGGAGCTGG TTCAGGAGAT CACACAACAT 180

TTATTCTTCT TACAGGTACA TCAGTCAAGG CTACCCCCCA GTTCTGAGAG AGAACTTGCC 240

CAGGAGTGGT TGCAGAGTTG GCCTCAGAGT TGACC 275

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 236 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GCTAAAGGGC TCAGAGTGCA GGCCGTGGGG CGCGAGGGTC CCGGGCCTGA GCCCCGCGCC 60

ATGGCCGGGG CCATCGCTTC CCGCATGAGC TTCAGCTCTC TCAAGAGGAA GCAACCCAAG 120

ACGTTCACCG TGAGGATCGT CACCATGGAC GCCGAGATGG AGTTCAATTG CGAGGTAACC 180

GGCCGGCAGC CCCGACTGCT GCGGTGACAG TCGAGGTGGA AGCTCGAGAG GTTCTC 236

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 188 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CCTCACTTCC CCTCACAGAG TATCATGTCT CCCTTGTTGC TCCTTTCAGG TAAAGAAGCA 60

GATTTTAGAT GAAAAGATCT ACTGCCCTCC TGAGGCTTCT GTGCTCCTGG CTTCTTACGC 120

CGTCCAGGCC AAGGTAGGCT CAAAGAAGAA AAATGTATTT TTNNCTGGGC GTTAATTTGG 180

GTCATGGG 188

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

TGGCAGTTAT CTTTAGAATC TCAATCGCCT GCTCTCCCTT TCTTCTTTCC AGTATGGTGA 60

CTACGACCCC AGTGTTCACA AGCGGGGATT TTTGGCCCAA GAGGAATTGC TTCCAAAAAG 120

GGTAAGAGAT TAAATTCCCT TTTCAGGAAG ACATAGCAGA TATGTGGTCT AAAAGAAAGC 180

TAACCAAAGG ACTTGAAGGA 200

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 256 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

TCTGTGTGAC TACTCCTGGT GTAGCTTTAA AATAGCTTTA CTGTTTGTAA AATGATGCAT 60

AATTATAAAA GTGGCAAACA ATACCAAATT TACTTCATGT GTAGGTTTTT TATTTTGCTC 120

TATTTTTTGG TAGGTAATAA ATCTGTATCA GATGACTCCG GAAATGTGGG AGGAGAGAAT 180

TACTGCTTGG TACGCAGAGC ACCGAGGCCG AGCCAGGTGA GGCCCATTCA TTGTTGGTTT 240

ACATTCCTTT ATGGGC 256

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GAATGCTTGA TTTGGTGGCC CACCCGCTCT CCACCCATCT CACTTAGCTC CAATGACAGT 60

GTCTTCCGTT CTCCCCACAG GGATGAAGCT GAAATGGAAT ATCTGAAGAT AGCTCAGGAC 120

CTGGAGATGT ACGGTGTGAA CTACTTTGCA ATCCGGGTGT GTTGAAACCT CTCTGAGCTC 180

CTTGTGTAGT AGACAGAGAC TGAGTGAGGG CCAGGACTGC TAAAATGGTT ACTTCTTCAT 240

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

TCTGTGGACC TGCTGAACTG CACATGTGAC AGTGTGTGCC AGATTCTTTG GAAGGTTGAA 60

TAAAATTTTG AGCCTCAGCT GGCGCTTACA GTAGCTGTTC TTATTGGATC CACAGAATAA 120

AAAGGGCACA GAGCTGCTGC TTGGAGTGGA TGCCCTGGGG CTTCACATTT ATGACCCTGA 180

AAACAGACTG ACCCCCAAGA TCTCCTTCCC GTGGAATGAA ATCCGAAACA TCTCGTACAG 240

TGACAAGGAG GTAGGACATG TGTGTACTGC AGATGGGTCC AGCAGATCTT TCCCTGTCTG 300

CCCCCCTCAC TGGAGCCTCC CCAGCCAGGG CATCTCCTTG TTATTCATAG AGTCCTTTAA 360

TTCCCAGGCT TTGAGGGTGT GGTTGTT 387

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GACTTGGTGC TCCTAATTCC CTGAGGTTTA GTGCCTGGAT ACTGGGAAGC CAGNACAAGG 60

GCATAACNTC ATGCTGGTCT GTGGCCAGTG TGGTTGCGCA TTTGTGGAAT TNCCAATTGC 120

TGGTAACATT CCAGGCTGTC GGACTGAAAC TGTGTTCTGC TTCATTCTTC CAGTTTACTA 180

TTAAACCACT GGATAAGAAA ATTGATGTCT TCAAGTTTAA CTCCTCAAAG CTTCGTGTTA 240

ATAAGCTGGT AAGTTGAGAT CCTGGTAAGT TGAGATCCTG GTTTTCATTA CTGATAATGG 300

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

TGCTACCTGC AAGAGCTCAA ACTGCTATGG CACTAGTGGG CCAGTAGGCA GTGAAGTAAA 60

TTTGTGGATA TTAACCTTTT TGTCTGCTTC TGTGGCCACA GATTCTCCAG CTATGTATCG 120

GGAACCATGA TCTATTTATG AGGAGAAGGA AAGCCGATTC TTTGGAAGTT CAGCAGATGA 180

AAGCCCAGGC CAGGGAGGAG AAGGCTAGAA AGCAGGTGAG CACAACCTTG TTTTAACTGA 240

TGATGTCACT GTGTGGTCAG 260

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 292 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

TCTTTGGCCC TTGTGGCACC CTAGGTCTCG AGCCCTGTGA TTCAATGACT GTTTTTCTTC 60

ACCCCTCGCA GATGGAGCGG CAGCGCCTCG CTCGAGAGAA GCAGATGAGG GAGGAGGCTG 120

AACGCACGAG GGATGAGTTG GAGAGGAGGC TGCTGCAGAT GAAAGAAGAA GCAACAATGG 180

CCAACGAAGC ACTGGTGATT TCTGAGGGGC TGGGGTTCCA GGAGGCTACT TGGGGACTTC 240

CTTGGCTTTT CTGGAGCTTG GTCTCCTGAA AACATGAGTT AGCAGCGTTT GC 292

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

CGGGAGAACA GCACATGATC CCACTTCAGC TAAGAGCACT GTGCCCTCCA GATGCGGTCT 60

GAGGAGACAG CTGACCTGTT GGCTGAAAAG GCCCAGATCA CCGAGGAGGA GGCAAAACTT 120

CTGGCCCAGA AGGCCGCAGA GGCTGAGCAG GAAATGCAGC GCATCAAGGC CACAGCGATT 180

CGCACGGAGG AGGAGAAGCG CCTGATGGAG CAGAAGGTGC TGGAAGCCGAG GTGCTGGCA 240

CTGAAGATGG CTGAGGAGTC AGAGAGGAGG TGAGGGGGCA CCGGGCACCA GACTGGCGAG 300

GAGGCTGGCG AAGGGCCGCA GACCAGCCTG CCCTGAGGCT GAGCTCTACA GCAGTTGTCC 360

TCAAG 365

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 227 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

GGTGTCTTTT CCTGCTACCT GCCCTCTTCT GTGAAGCTGA CATCTCATCC TTTCCTTGCA 60

GGGCCAAAGA GGCAGATCAG CTGAAGCAGG ACCTGCAGGA AGCACGCGAG GCGGAGCGAA 120

GAGCCAAGCA GAAGCTCCTG GAGATTGCCA CCAAGCCCAC GTACCCGGTG AGCCTGGGGG 180

CCACCAGCTG GGGCTGCCTT AGTCCTGGTG ATGTTCTCTT TCCTCCC 227

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

TGTGCCATTG CCTCTGTGGC TGCTGGAGGA TCGGTTGTCA ACACAGTAGT GTCCTTCTGT 60

GCTTGTATGA CCCAAGCTCC TAATCCGAAA TTTCTCATTA ACAGCCCATG AACCCAATTC 120

CAGCACCGTT GCCTCCTGAC ATACCAAGCT TCAACCTCAT TGGTGACAGC CTGTCTTTCG 180

ACTTCAAAGA TACTGACATG AAGCGGCTTT CCATGGAGAT AGAGAAAGAA AAGTATGTAG 240

CCCCCTGTGC CCTGCTGTGG GCTTGCTGTG AACTAGACTG A 281

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 335 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

TGGCCAAGTA GAGACGTGAN NCCAGCNTNA AACCCTAGAT CGCACACCAA GCAGCTTGTG 60

GGCCACAGAG CACCTGAGCC GTGTCTCACT GTCTGCCCAA GCCCTGATGC ATGATACCCT 120

CTTGCCGGCA GAGTGGAATA CATGGAAAAG AGCAAGCATC TGCAGGAGCA GCTCAATGAA 180

CTCAAGACAG AAATCGAGGC CTTGAAACTG AAAGAGAGGG AGACAGCTCT GGATATTCTG 240

CACAATGAGA ACTCCGACAG GGGTGGCAGC AGCAAGCACA ATACCATTAA AAAGGTACCC 300

AGGGTCTCTT TCTTGTATTT TGCTGATCAG GACCA 335

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 254 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

CAAACAAAAT CACTCATCAC GATNTCAGGC CTATCCAAGC ATTTTGCANA TGGCACTTAT 60

GGCATTGTTG ATATCACAGG GTATGTTTTT GTTTTTCTTC ATTTTATTTT GCTGGTTTAG 120

CCTCAAGCCC AAGGCAGAAG ACCTATCTGC ATTTGAGCCC TCAAAGTAGC TTGTTCCCAG 180

GTACTCTCTA TGTGGTGATG GTGCTGCCCT CTGTGATACT AACCCGTGCA TGAGNTTGCC 240

TGTCTCTGTC TCGG 254

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

AGGACCCTGT GAGACAGAGC GGAGGTCNNG TGCCCTCTCA GCTTCTTCTC TGCTTTCTTA 60

CAGCTCACCT TGCAGAGCGC CAAGTCCCGA GTGGCCTTCT TTGAAGAGCT CTAGCAGGTG 120

ACCCAGCCAC CCCAGGACCT GCCACTTCTC CTGCTACCGG GACCGCGGGA TGGACCAGAT 180

ATCAAGAGAG CCATCCATAG GGAGCTGGCT GGGGGTTTCC GTGGGAGCTC CAGAACTTTC 240

CCCAGCTGAG TGAAGAGCCC AGCCCCTCTT ATGTGCAATT GCCTTGAACT ACGACCCTGT 300

AGAGATTTCT CTCATGGCGT TCTAGTTCTC TGACCTGAG 339

Claims

1. An isolated, purified or recombinant nucleic acid sequence comprising:

(a) a *merlin* structural gene as shown in Figure 3 or a functional derivative thereof or its complementary strand;

(b) a sequence homologous to, or which hybridises to either sequence in (a) under stringent conditions; or

(c) is homologous to, or would hybridise under stringent conditions to, a sequence in (a) or (b) but for the degeneracy of the genetic code; or

(d) an oligonucleotide specific for any of (a), (b) or (c)

(a) a *merlin* gene containing the trapped exons are shown in Figure 3

(b) a sequence homologous to, or which hybridises to either sequence in (a) under stringent conditions; or

(c) is homologous to, or would hybridise under stringent conditions to, a sequence in (a) or (b) but for the degeneracy of the genetic code, or

(d) an oligonucleotide specific for any of (a), (b) or (c).

3. A nucleic acid sequence comprising a mutant *merlin* gene wherein the mutation in the gene is as shown in Table 3 or in Table 4 in the sequence according to Figure 3.

4. A nucleic acid sequence comprising a mutant *merlin* gene wherein the mutation in the gene is in a sequence as defined in claim 1 or claim 2.

5. A nucleic acid sequence comprising a mutant *merlin* gene wherein there is an A → T transversion at the first base of amino acid 220 according to Figure 3.

6. A nucleic acid sequence according to any preceding claim wherein the nucleic acid encoding *merlin* is operably linked to transcriptional and/or translational expression signals.

7. A vector comprising the nucleic acid sequence of any preceding claim.

8. A host cell transformed or transfected with a vector according to claim 7.

9. A host cell according to claim 8 which is the *E. coli* cell line deposited under Accession No. ATCC 75509.

10. An isolated, purified or recombinant polypeptide comprising a *merlin* protein having the amino acid sequence shown in Figure 3 and/or is the protein expressed by the *E. coli* cell line deposited under Accession No. ATCC 75509, or a mutant or variant thereof having *merlin* protein activity.

11. An isolated, purified or recombinant polypeptide comprising a *merlin* protein having an amino acid sequence as shown in Figure 7, or a mutant or variant thereof.

12. An anti-*merlin* antibody raised against a polypeptide defined in claim 10 or claim 11, optionally labelled.

13. A method of detecting a *merlin*-associated tumour and/or disease or neurofibromatosis-2 (NF2) in a patient suspected of having or having predisposition to, said disorders, the method comprising detecting the presence of and/or evaluating the characteristics of *merlin* DNA or *merlin* mRNA comprising a nucleic acid sequence as defined in any one of claims 1 to 5, and/or *merlin* protein comprising a polypeptide as defined in claim 10 or claim 11, in a sample taken from the patient.

14. A method according to claim 13 which comprises detecting and/or evaluating whether the *merlin* DNA is deleted, missing, mutated, aberrant or not expressing normal *merlin* protein.

15. A method according to claim 13 which comprises:

(a) taking a biological, tissue or biopsy sample from the patient;

(b) detecting the presence of and/or evaluating the characteristics of *merlin* DNA, merlin mRNA and/or *merlin* protein in the sample to obtain a first set of results;

(c) comparing the first set of results with a second set of results obtained using the same or similar methodology for an individual not suspected of having said disorders; and

(d) if the first and second set of results differ in that the *merlin* DNA is deleted, missing, aberrant, mutated or

16. A method according to claim [illegible] which comprises

(a) taking a tissue, biological or biopsy sample from the patient;

(b) detecting the presence of and/or evaluating the characteristics of *merlin* DNA, merlin mRNA and/or *merlin* protein in the sample to obtain a first set of results;

(c) comparing the first set of results with a second set of results obtained using the same or similar methodology for evaluations in an individual known to have at least one of said disorders; and

(d) if the first and second set of results are identical, this indicates that the *merlin* DNA in the patient is deleted, mutated or not expressing normal *merlin* protein.

17. A method according to either claim 15 or 16 wherein the second set of results is obtained using *merlin* DNA containing a mutation at the first position of amino acid 220 according to Figure 3 which is an A → T transversion.

18. A method according to any of claims 13 to 17 wherein the detection or evaluation is by a Southern blot procedure using DNA from the sample digested with the restriction enzyme *Rsa*I or an antibody according to claim 11.

19. A method of characterising a mutation in a subject suspected of having a mutation in the *merlin* gene comprising a nucleic acid sequence as defined in any one of claims 1 to 5, the method comprising:

(a) amplifying each of the exons in the *merlin* gene of the subject,

(b) denaturing the complementary strands of the amplified exons;

(c) diluting the denatured separate, complementary strands to allow each single-stranded DNA molecule to assume a secondary structure conformation;

(d) subjecting the DNA molecules to electrophoresis under non-denaturing conditions;

(e) comparing the electrophoresis pattern of the single-stranded molecules with the electrophoresis pattern of single-stranded molecules containing the same amplified exon from a control individual which has either a normal or NF2 heterozygous genotype; and

(f) sequencing any amplification product which has an electrophoretic pattern different from the pattern obtained from the DNA of the control individual.

20. A vector as defined in claim 6, or a nucleic acid sequence as defined in any one of claims 1 to 3, encoding a functional *merlin* protein, or a functional *merlin* polypeptide as defined in claim 9, for use in gene therapy.

21. The use of a vector as defined in claim 7, or a nucleic acid sequence as defined in any one of claims 1 to 5, encoding a functional *merlin* protein, or a functional *merlin* polypeptide as defined in claim 10, in the preparation of an agent for the treatment or prevention of a *merlin*-associated tumour or disease or neurofibromatosis-2 (NF2).

22. The use according to claim 21 wherein the treatment or prevention is for schwannoma or meningioma (either being, for example, vestibular, cranial nerve or spinal nerve root), posterior capsular lens opacities, deafness or hearing loss, balance disorders or paralysis.

23. A pharmaceutical composition, suitably adapted for injection, comprising a vector as defined in claim 7, or a nucleic acid as defined in any one of claims 1 to 5, encoding a functional *merlin* protein, a functional *merlin* polypeptide as defined in claim 10 or host as defined in claim 8 expressing a functional protein, or a combination thereof, and a pharmaceutically acceptable carrier.

Patentansprüche

a) einem [illegible]-Strukturgen wie dargestellt in Figur 1 oder einem funktionellen Derivat davon oder seinem Komplementärstrang,

(b) einer Sequenz, die zu einer Sequenz in (a) homolog ist oder damit unter stringenten Bedingungen hybridisiert; oder

(c) die zu einer Sequenz in (a) oder (b) homolog ist oder ohne die Degeneriertheit des genetischen Codes unter stringenten Bedingungen damit hybridisieren würde; oder

(d) einem für (a), (b) oder (c) spezifischen Oligonucleotid.

2. Isolierte, aufgereinigte oder rekombinante Nucleinsäuresequenz mit:

(a) einem *merlin*-Gen mit den in Figur 5 gezeigten gefangenen Exons;

(b) einer Sequenz, die zu einer Sequenz in (a) homolog ist oder damit unter stringenten Bedingungen hybridisiert; oder

(c) die zu einer Sequenz in (a) oder (b) homolog ist oder ohne die Degeneriertheit des genetischen Codes unter stringenten Bedingungen damit hybridisieren würde; oder

(d) einem für (a), (b) oder (c) spezifischen Oligonucleotid.

3. Nucleinsäuresequenz mit einem mutierten *merlin*-Gen, wobei die Mutation des Gens, wie gezeigt in Tabelle 3 oder Tabelle 4, in der Sequenz gemäß Figur 3 ist.

4. Nucleinsäuresequenz mit einem mutierten *merlin*-Gen, wobei die Mutation des Gens sich in einer Sequenz nach Anspruch 1 oder Anspruch 2 befindet.

5. Nucleinsäuresequenz mit einem mutierten *merlin*-Gen, in dem sich eine A › T Transversion an der 1. Base der Aminosäure 220 gemäß Figur 3 befindet.

6. Nucleinsäuresequenz gemäß einem der vorhergehenden Ansprüche, wobei die für *merlin* kodierende Nucleinsäure operativ an transkriptonelle und/oder translationelle Expressionssignale gekoppelt ist.

7. Vektor mit einer Nucleinsäuresequenz nach einem der vorhergehenden Ansprüche.

8. Wirtszelle, mit einem Vektor gemäß Anspruch 7 transformiert oder transfiziert.

9. Wirtszelle gemäß Anspruch 8, weiche die *E.coli*-Zellinie ist, die unter Zugangsnummer ATCC 75509 hinterlegt ist.

10. Isoliertes, aufgereinigtes oder rekombinantes Polypeptid, das ein *merlin*-Protein enthält, welches die in Figur 3 gezeigte Aminosäuresequenz hat und/oder das Protein ist, welches von der *E.coli*-Zellinie exprimiert wird, die unter Zugangsnummer ATCC 75509 hinterlegt ist oder eine Mutante oder eine Variante davon mit *merlin*-Proteinaktivität.

11. Isoliertes, aufgereinigtes oder rekombinantes Polypeptid, mit einem *merlin*-Protein, welches eine Aminosäuresequenz gemäß Figur 7 hat, oder eine Mutante oder Variante davon.

12. Anti-*merlin* Antikörper, der gegen ein in Anspruch 10 oder Anspruch 11 definiertes Polypeptid gebildet wurde.

13. Verfahren zur Detektion eines *merlin*-assoziierten Tumors und/oder einer *merlin*-assoziierten Krankheit oder Neurofibromatose-2 (NF2) in einem Patienten mit Verdacht auf diese Störungen oder mit Verdacht auf die Prädisposition für diese Störungen durch die Detektion der Gegenwart und/oder die Bewertung der Eigenschaften von *merlin*-DNA oder *merlin*-mRNA mit einer in einem der Ansprüche 1 bis 5 definierten Nucleinsäuresequenz und/oder von *merlin*-Protein mit einem in einem der Ansprüche 10 oder 11 definierten Polypeptid in einer dem Pati-

[illegible] fehlt, mutiert oder aberrant ist oder kein normales [illegible]-Protein exprimiert

15. Verfahren gemäß Anspruch 13 mit:

(a) der Entnahme einer biologischen, Gewebe- oder Biopsieprobe vom Patienten;

(b) der Detektion der Gegenwart und/oder der Bewertung der Eigenschaften von *merlin*-DNA, *merlin*-mRNA und/oder *merlin*-Protein in der Probe zum Erhalt eines 1. Satzes von Ergebnissen;

(c) dem Vergleich des 1. Satzes von Ergebnissen mit einem 2. Satz von Ergebnissen, der durch Verwendung derselben oder einer ähnlichen Methodologie für ein Individuum erhalten wurde, welches nicht im Verdacht steht, besagte Störungen zu haben; wobei

(d) falls der 1. und 2. Satz von Ergebnissen sich darin unterscheiden, daß die *merlin*-DNA deletiert ist, fehlt, aberrant ist, mutiert ist oder kein *merlin*-Protein exprimiert, dies die Gegenwart dieser Störungen oder die Prädisposition oder die Tendenz des Patienten anzeigt, diese Störungen zu entwickeln.

16. Verfahren gemäß Anspruch 13 mit:

(a) der Entnahme einer Gewebe-, biologischen- oder Biopsieprobe von dem Patienten;

(b) der Detektion der Gegenwart und/oder dem Bewerten der Eigenschaften von *merlin*-DNA, *merlin*-mRNA und/oder *merlin*-Protein in der Probe um einen 1. Satz von Ergebnissen zu erhalten;

(c) dem Vergleich des 1. Satzes von Ergebnissen mit einem 2. Satz von Ergebnissen, der durch Verwendung derselben oder ähnlicher Methodologie für Bewertungen in einem Individuum erhalten wurde, von dem bekannt ist, daß es mindestens eine der genannten Störungen aufweist; wobei

(d) falls der 1. und 2. Satz von Ergebnissen identisch sind, dies anzeigt, daß die *merlin*-DNA in dem Patienten deletiert oder mutiert ist oder kein normales *merlin*-Protein exprimiert.

17. Verfahren nach einem der Ansprüche 15 oder 16, wobei der 2. Satz von Ergebnissen durch die Verwendung einer *merlin*-DNA erhalten wird, die eine Mutation an der 1. Position von Aminosäure 220 gemäß Figur 3 enthält, welche eine A → T Transversion ist.

18. Verfahren gemäß einem der Ansprüche 13 bis 17, wobei die Detektion oder Bewertung durch ein Southern Bot Verfahren unter Verwendung von mit dem Restriktionsenzym RsaI verdauter DNA aus der Probe oder durch einem Antikörper gemäß Anspruch 11 vorgenommen wird.

19. Verfahren zur Charakterisierung einer Mutation in einem Wesen, welches unter Verdacht steht, eine Mutation mit einer Nucleinsäuresequenz gemäß einem der Ansprüche 1 bis 5 in dem *merlin*-Gen zu haben durch:

(a) Amplifikation jedes der Exons im *merlin*-Gen des Wesens;

(b) Denaturierung der Komplementärstränge der amplifizierten Exons;

(c) Verdünnung der denaturierten, separaten Komplementärstränge, um es jedem einzelsträngigen DNA-Molekül zu erlauben, eine Sekundärstrukturkonformation einzunehmen.

(d) Elektrophorese der DNA-Moleküle unter nicht-denaturierenden Bedingungen;

(e) Vergleich des Elektrophoresemusters der einzelsträngigen Moleküle mit dem Elektrophoresemuster von einzelsträngigen Molekülen, die dasselbe amplifizierte Exon von einem Kontrollindividium enthalten, welches entweder einen normalen oder einen heterozygoten NF2-Genotyp hat;

(f) Bestimmung jedes Amplifikationsproduktes, das ein Elektrophoresemuster aufweist, welches sich von

[illegible] Vektor wie in Anspruch 6 definiert, oder Nucleinsäuresequenz wie in einem der Ansprüche 1 bis 5 definiert, [illegible] oder die für ein funktionelles *merlin*-Protein oder für ein funktionelles *merlin*-Polypeptid wie in Anspruch 9 definiert.

kodiert, zur Verwendung in der Gentherapie.

21. Verwendung eines Vektors, wie in Anspruch 7 definiert oder einer Nucleinsäuresequenz, wie in einem der Ansprüche 1 bis 5 definiert, der oder die für ein funktionelles *merlin*-Protein oder *merlin*-Polypeptid wie in Anspruch 10 definiert, kodiert, zur Herstellung eines Mittels für die Behandlung oder Vorbeugung eines *merlin*-assoziierten Tumors oder einer *merlin*-assoziierten Krankheit oder Neurofibromatose-2(NF2).

22. Verwendung gemäß Anspruch 21, wobei die Behandlung oder Vorbeugung Schwannome oder Meningiome (beispielsweise vestibuläre, von Himnerven oder Spinalnervenwurzeln stammende), posteriore kapsuläre Linsentrübungen, Taubheit oder Verlust des Hörvermögens, Gleichgewichtsstörungen oder Paralyse behilft.

23. Pharmazeutische Zusammensetzung, geeignet für die Injektion, mit einem Vektor wie in Anspruch 7 definiert, oder einer Nucleinsäure, wie in einem der Ansprüche 1 bis 5 definiert, der oder die für ein funktionelles *merlin*-Protein oder ein funktionelles *merlin*-Polypeptid, wie in Anspruch 10 definiert, kodiert oder einem Wirt, wie in Anspruch 8 definiert, der ein funktionelles Protein exprimiert oder eine Kombination davon und einem pharmazeutisch akzeptablen Träger.

Revendications

1. Séquence d'acide nucléique, isolée, purifiée ou recombinante, comprenant :

   (a) un gène de structure *merlin* tel que représenté sur la Figure 3 ou un dérivé fonctionnel de celui-ci ou son brin complémentaire ;

   (b) une séquence homologue de, ou qui s'hybride à, l'une ou l'autre des séquences indiquées en (a) dans des conditions stringentes ; ou

   (c) est homologue de, ou s'hybriderait dans des conditions stringentes à, une séquence indiquée en (a) ou (b) mais pour la dégénérescence du code génétique ; ou

   (d) un oligonucléotide spécifique pour l'un quelconque parmi (a), (b) et (c).

2. Séquence d'acide nucléique, isolée, purifiée ou recombinante, comprenant :

   (a) un gène *merlin* contenant les exons piégés tels que représentés sur la Figure 5 ;

   (b) une séquence homologue de, ou qui s'hybride à, l'une ou l'autre des séquences indiquées en (a) dans des conditions stringentes ; ou

   (c) est homologue de, ou s'hybriderait dans des conditions stringentes à, une séquence indiquée en (a) ou (b) mais pour la dégénérescence du code génétique ; ou

   (d) un oligonucléotide spécifique pour l'un quelconque parmi (a), (b) ou (c).

3. Séquence d'acide nucléique comprenant un gène *merlin* mutant, dans laquelle la mutation dans le gène est telle que representee dans le Tableau 3 ou le Tableau 4 dans la séquence selon la Figure 3.

4. Séquence d'acide nucléique comprenant un gène *merlin* mutant, dans laquelle la mutation dans le gène est dans une séquence telle que définie à la revendication 1 ou la revendication 2.

5. Séquence d'acide nucléique comprenant un gène *merlin* mutant, dans laquelle il y a une tranversion A -> T au niveau de la première base de l'acide aminé 220 selon la Figure 3.

6. Séquence d'acide nucléique selon l'une quelconque des revendications précédentes, dans laquelle l'acide nucléi-

[illegible] comprenant [illegible]

8. Cellule hôte transformée ou transfectee par un vecteur tel que défini à la revendication 7.

9. Cellule hôte selon la revendication 8, qui est la lignée cellulaire d'*E. coli* déposée sous le No. d'accès ATCC 75509.

10. Polypeptide isolé, purifié ou recombinant, comprenant une protéine *merlin* ayant la séquence d'acides aminés représentee sur la Figure 3 et/ou qui est la protéine exprimée par la lignée cellulaire d'*E. coli* déposée sous le No. d'accès ATCC 75509, ou un mutant ou variant de celle-ci ayant une activité de protéine *merlin*.

11. Polypeptide isolé, purifié ou recombinant, comprenant une protéine *merlin* ayant une séquence d'acides aminés telle que représentée sur la Figure 7, ou un mutant ou variant de celle-ci.

12. Anticorps anti-*merlin* dirigé contre un polypeptide défini à la revendication 10 ou la revendication 11, facultativement marqué

13. Procédé de détection d'une tumeur et/ou maladie associée à *merlin* ou de la neurofibromatose-2 (NF2) chez un patient suspecté d'avoir lesdits, ou ayant une prédisposition auxdits, troubles, le procédé comprenant la détection de la présence, et/ou l'évaluation des caractéristiques, de l'ADN merlin ou de l'ARNm *merlin* comprenant une séquence d'acide nucléique telle que définie à l'une des revendications 1 à 5, et/ou d'une protéine *merlin* comprenant un polypeptide tel que défini à la revendication 10 ou à la revendication 11, dans un échantillon prélevé sur le patient.

14. Procédé selon la revendication 13, qui comprend la détection et/ou l'évaluation du fait de savoir si l'ADN *merlin* est supprimé, est manquant, a muté, est aberrant ou n'exprime pas la protéine *merlin* normale.

15. Procédé selon la revendication 13, qui comprend les opérations consistant à :

(a) prélever un échantillon de tissu, biologique ou de biopsie chez un patient ;

(b) détecter la présence, et/ou évaluer les caractéristiques, de l'ADN *merlin*, de l'ARNm *merlin* et/ou de la protéine *merlin* dans l'échantillon, afin d'obtenir un premier ensemble de résultats ;

(c) comparer le premier ensemble de résultats avec un second ensemble de résultats obtenu à l'aide de la même méthodologie ou d'une méthodologie analogue chez un individu non suspecté d'avoir de tels troubles ; et

(d) si les premier et second ensembles de résultats diffèrent par le fait que l'ADN *merlin* comporte une délétion, est manquant, est aberrant, a muté ou n'exprime pas la protéine *merlin*, ceci indique alors la présence, la prédisposition ou la tendance du patient à développer lesdits troubles.

16. Procédé selon la revendication 13, qui comprend les opérations consistant à :

(a) prélever un échantillon de tissu, biologique ou de biopsie sur un patient ;

(b) detecter la présence, et/ou évaluer les caractéristiques, de l'ADN *merlin*, de l'ARNm *merlin* et/ou de la protéine *merlin* dans l'échantillon, afin d'obtenir un premier ensemble de résultats ;

(c) comparer le premier ensemble de résultats avec un second ensemble de résultats obtenu à l'aide de la même méthodologie ou d'une méthodologie analogue pour des évaluations chez un individu connu pour avoir au moins l'un desdits troubles ; et

(d) si les premier et second ensembles de résultats sont identiques, ceci indique que l'ADN *merlin* chez le patient comporte une délétion, a muté ou n'exprime pas la protéine *merlin* normale.

17. Procédé selon l'une des revendications 15 et 16, dans lequel le second ensemble de résultats est obtenu à l'aide

18. Procédé selon l'une des revendications 13 à 17, dans lequel la détection ou l'évaluation s'effectue par ... moyen

operatoire de Southern-blot à l'aide d'ADN provenant de l'échantillon digéré par l'enzyme de restriction *Rsa*I ou un anticorps selon la revendication 12.

19. Procédé de caractérisation d'une mutation chez un sujet suspecté d'avoir une mutation dans le gène *merlin* comprenant une séquence d'acide nucléique telle que définie à l'une quelconque des revendications 1 à 5, le procédé comprenant les opérations consistant à :

(a) amplifier chacun des exons dans le gène *merlin* du sujet ;

(b) dénaturer les brins complémentaires des exons amplifiés ;

(c) diluer les brins complémentaires séparés, dénaturés, pour permettre à chaque molécule d'ADN monocaténaire d'adopter une conformation de structure secondaire ;

(d) soumettre les molécules d'ADN à une électrophorèse dans des conditions non dénaturantes ;

(e) comparer le diagramme d'électrophorèse des molécules monocaténaires avec le diagramme d'électrophorèse de molécules monocaténaires contenant le même exon amplifié provenant d'un individu témoin qui a un génotype soit normal soit hétérozygote pour NF2 ; et

(f) séquencer tout produit d'amplification qui a un diagramme électrophorétique différent du diagramme obtenu à partir de l'ADN de l'individu témoin.

20. Vecteur tel que défini à la revendication 7, ou séquence d'acide nucléique telle que définie à l'une des revendications 1 à 3, codant pour une protéine *merlin* fonctionnelle ou un polypeptide *merlin* fonctionnel tel que défini à la revendication 10, pour une utilisation en thérapie génique.

21. Utilisation d'un vecteur tel que défini à la revendication 7, ou d'une séquence d'acide nucléique telle que définie à l'une des revendications 1 à 5, codant pour une protéine *merlin* fonctionnelle ou un polypeptide *merlin* fonctionnel tel que défini à la revendication 10, dans la préparation d'un agent pour le traitement ou la prévention d'une tumeur ou maladie associée à *merlin* ou de la neurofibromatose-2 (NF2).

22. Utilisation selon la revendication 21, dans laquelle le traitement ou la prévention est pour le schwannome ou le méningiome (l'une ou l'autre étant, par exemple, vestibulaire, des nerfs crâniens ou de la racine des nerfs rachidiens), les opacités du cristallin capsulaire postérieur, la surdité ou la perte de l'audition, les troubles de l'équilibre ou la paralysie.

23. Composition pharmaceutique, adaptée de façon appropriée pour l'injection, comprenant un vecteur tel que défini à la revendication 7, ou un acide nucléique tel que défini à l'une des revendications 1 à 5, codant pour une protéine *merlin* fonctionnelle, un polypeptide *merlin* fonctionnel tel que défini à la revendication 10 ou un hôte tel que défini à la revendication 8 exprimant une protéine fonctionnelle, ou une combinaison de ceux-ci, et un support pharmaceutiquement acceptable.

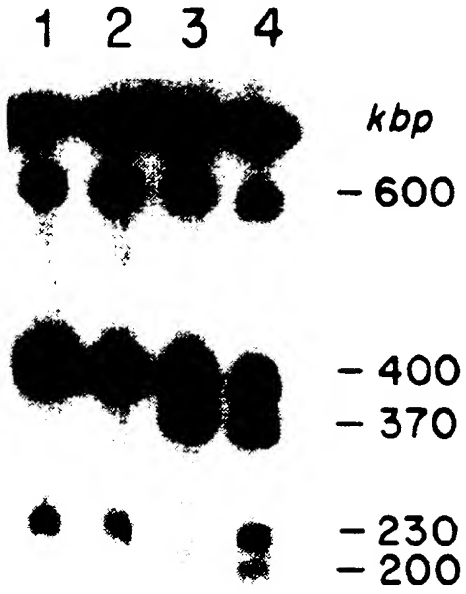


FIG. 1

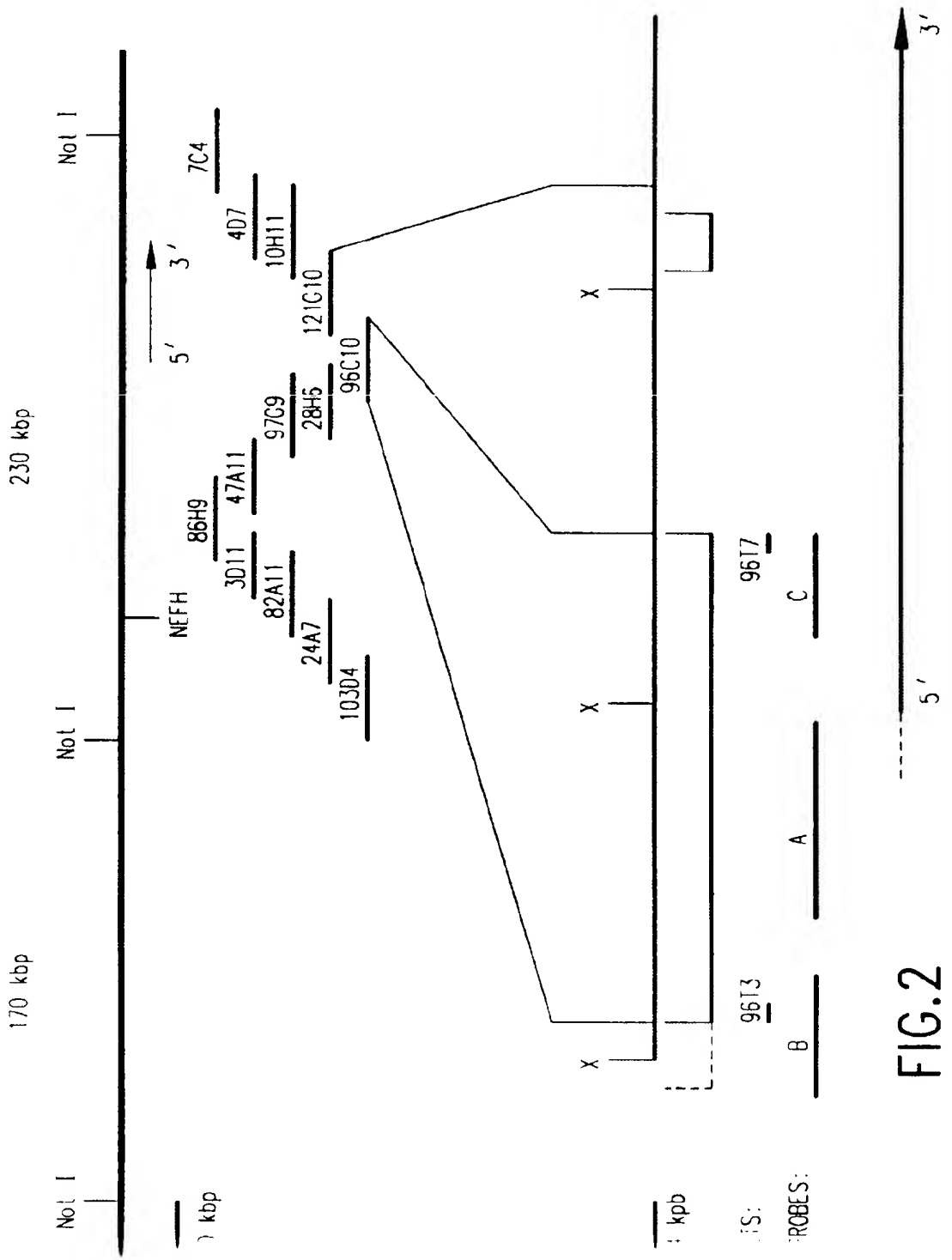

Not I
230 kbp
Not I
170 kbp
Not I
NEFH
86H9
3D11
47A11
82A11
24A7
10304
97C9
28H5
96C10
121C10
10H11
4D7
7C4
5'
3'
X
X
X
9613
9617
A
B
C
5'
3'

FIG.2

```
ACGGCAGCCG TCAGGGACCG TCCCCCAACT CCCCTTTCCG CTCAGGCAGG GTCCTCGCGG  60

CCCATGCTGG CCGCTGGGGA CCCGCGCAGC CCAGACCGTT CCCGGGCCGG CCAGCCGGCA 120

CCATCGTGGC CCTGAGGCCT GTGCAGCAAC TCCAGGGGGG CTAAAGGGCT CAGAGTGCAG 180

GCCGTGGGGC GCGAGGGTCC CGGGCCTGAG CCCCGCGCC ATG GCC GGG GCC ATC  234

                                          Met Ala Gly Ala Ile
                                            1               5

GCT TCC CGC ATG AGC TTC AGC TCT CTC AAG AGG AAG CAA CCC AAG ACG  282

Ala Ser Arg Met Ser Phe Ser Ser Leu Lys Arg Lys Gln Pro Lys Thr
                10                  15                  20

TTC ACC GTG AGG ATC GTC ACC ATG GAC GCC GAG ATG GAG TTC AAT TGC  330

Phe Thr Val Arg Ile Val Thr Met Asp Ala Glu Met Glu Phe Asn Cys
            25                  30                  35

GAG ATG AAG TGG AAA GGG AAG GAC CTC TTT GAT TTG GTG TGC CGG ACT  378

Glu Met Lys Trp Lys Gly Lys Asp Leu Phe Asp Leu Val Cys Arg Thr
        40                  45                  50

CTG GGG CTC CGA GAA ACC TGG TTC TTT GGA CTG CAG TAC ACA ATC AAG  426

Leu Gly Leu Arg Glu Thr Trp Phe Phe Gly Leu Gln Tyr Thr Ile Lys
    55                  60                  65

GAC ACA GTG GCC TGG CTC AAA ATG GAC AAG AAG GTA CTG GAT CAT GAT  474

Asp Thr Val Ala Trp Leu Lys Met Asp Lys Lys Val Leu Asp His Asp
70                  75                  80                  85
```

FIG.3A

```
GTT TCA AAG GAA GAA CCA GTC ACC TTT CAC TTC TTG GCC AAA TTT TAT 522

Val Ser Lys Glu Glu Pro Val Thr Phe His Phe Leu Ala Lys Phe Tyr
                 90                  95                 100

CCT GAG AAT GCT GAA GAG GAC CTG GTT CAG GAG ATC ACA CAA CAT TTA 570

Pro Glu Asn Ala Glu Glu Glu Leu Val Gln Glu Ile Thr Gln His Leu
            105                 110                 115

TTC TTC TTA CAG GTA AAG AAG CAG ATT TTA GAT GAA AAG ATC TAC TGC 618

Phe Phe Leu Gln Val Lys Lys Gln Ile Leu Asp Glu Lys Ile Tyr Cys
        120                 125                 130

CCT CCT GAG GCT TCT GTG CTC CTG GCT TCT TAC GCC GTC CAG GCC AAG 666

Pro Pro Glu Ala Ser Val Leu Leu Ala Ser Tyr Ala Val Gln Ala Lys
    135                 140                 145

TAT GGT GAC TAC GAC CCC AGT GTT CAC AAG CGG GGA TTT TTG GCC CAA 714

Tyr Gly Asp Tyr Asp Pro Ser Val His Lys Arg Gly Phe Leu Ala Gln
150                 155                 160                 165

GAG GAA TTG CTT CCA AAA AGG GTA ATA AAT CTG TAT CAG ATG ACT CCG 762

Glu Glu Leu Leu Pro Lys Arg Val Ile Asn Leu Tyr Gln Met Thr Pro
                170                 175                 180

GAA ATG TGG GAG GAG AGA ATT ACT GCT TGG TAC GCA GAG CAC CGA GGC 810

Glu Met Trp Glu Glu Arg Ile Thr Ala Trp Tyr Ala Glu His Arg Gly
            185                 190                 195

CGA GCC AGG GAT GAA GCT GAA ATG GAA TAT CTG AAG ATA GCT CAG GAC 858

Arg Ala Arg Asp Glu Ala Glu Met Glu Tyr Leu Lys Ile Ala Gln Asp
        200                 205                 210
```

FIG.3B

```
CTG GAG ATG TAC GGT GTG AAC TAC TTT GCA ATC CGG AAT AAA AAG GGC 906

Leu Glu Met Tyr Gly Val Asn Tyr Phe Ala Ile Arg Asn Lys Lys Gly
    215                 220                 225

ACA GAG CTG CTG CTT GGA GTG GAT GCC CTG GGG CTT CAC ATT TAT GAC 954

Thr Glu Leu Leu Leu Gly Val Asp Ala Leu Gly Leu His Ile Tyr Asp
230                 235                 240                 245

CCT GAG AAC AGA CTG ACC CCC AAG ATC TCC TTC CCG TGG AAT GAA ATC 1002

Pro Glu Asn Arg Leu Thr Pro Lys Ile Ser Phe Pro Trp Asn Glu Ile
                250                 255                 260

CGA AAC ATC TCG TAC AGT GAC AAG GAG TTT ACT ATT AAA CCA CTG GAT 1050

Arg Asn Ile Ser Tyr Ser Asp Lys Glu Phe Thr Ile Lys Pro Leu Asp
            265                 270                 275

AAG AAA ATT GAT GTC TTC AAG TTT AAC TCC TCA AAG CTT CGT GTT AAT 1098

Lys Lys Ile Asp Val Phe Lys Phe Asn Ser Ser Lys Leu Arg Val Asn
        280                 285                 290

AAG CTG ATT CTC CAG CTA TGT ATC GGG AAC CAT GAT CTA TTT ATG AGG 1146

Lys Leu Ile Leu Gln Leu Cys Ile Gly Asn His Asp Leu Phe Met Arg
    295                 300                 305

AGA AGG AAA GCC GAT TCT TTG GAA GTT CAG CAG ATG AAA GCC CAG GCC 1194

Arg Arg Lys Ala Asp Ser Leu Glu Val Gln Gln Met Lys Ala Gln Ala
310                 315                 320                 325

AGG GAG GAG AAG GCT AGA AAG CAG ATG GAG CGG CAG CGC CTC GCT CGA 1242

Arg Glu Glu Lys Ala Arg Lys Gln Met Glu Arg Gln Arg Leu Ala Arg
                330                 335                 340
```

FIG.3C

```
GAG AAG CAG ATG AGG GAC GAG GCT GAA CGC ACG AGG GAT GAC TTG GAG 1290

Glu Lys Gln Met Arg Glu Glu Ala Glu Arg Thr Arg Asp Glu Leu Glu
            345                 350                 355

AGG AGC CTG CTG CAG ATG AAA GAA GAA GCA ACA ATG GCC AAC GAA GCA 1338

Arg Arg Leu Leu Gln Met Lys Glu Glu Ala Thr Met Ala Asn Glu Ala
        360                 365                 370

CTG ATG CGG TCT GAG GAC ACA GCT GAC CTG TTG GCT GAA AAG GCC CAG 1386

Leu Met Arg Ser Glu Glu Thr Ala Asp Leu Leu Ala Glu Lys Ala Gln
    375                 380                 385

ATC ACC GAG GAG GAG GCA AAA CTT CTG GCC CAG AAG GCC GCA GAG GCT 1434

Ile Thr Glu Glu Glu Ala Lys Leu Leu Ala Gln Lys Ala Ala Glu Ala
390                 395                 400                 405


GAG CAG GAA ATG CAG CGC ATC AAG GCC ACA GCG ATT CGC ACG GAG GAG 1482

Glu Gln Glu Met Gln Arg Ile Lys Ala Thr Ala Ile Arg Thr Glu Glu
                410                 415                 420

GAG AAG CGC CTG ATG GAG CAG AAG GTG CTG GAA GCC GAG GTG CTG GCA 1530

Glu Lys Arg Leu Met Glu Gln Lys Val Leu Glu Ala Glu Val Leu Ala
            425                 430                 435

CTG AAG ATG GCT GAG GAG TCA GAG AGG AGG GCC AAA GAG GCA GAT CAG 1578

Leu Lys Met Ala Glu Glu Ser Glu Arg Arg Ala Lys Glu Ala Asp Gln
        440                 445                 450

CTG AAG CAG GAC CTG CAG GAA GCA CGC GAG GCG GAG CGA AGA GCC AAG 1626

Leu Lys Gln Asp Leu Gln Glu Ala Arg Glu Ala Glu Arg Arg Ala Lys
    455                 460                 465
```

FIG.3D

```
CAG AAG CTC CTG GAG ATT GCC ACC AAG CCC ACG TAC CCG CCC ATG AAC 1674

Gln Lys Leu Leu Glu Ile Ala Thr Lys Pro Thr Tyr Pro Pro Met Asn
470                 475                 480                 485

CCA ATT CCA GCA CCG TTG CCT CCT GAC ATA CCA AGC TTC AAC CTC ATT 1722

Pro Ile Pro Ala Pro Leu Pro Pro Asp Ile Pro Ser Phe Asn Leu Ile
                490                 495                 500

GGT GAC AGC CTG TCT TTC GAC TTC AAA GAT ACT GAC ATG AAG CGG CTT 1770

Gly Asp Ser Leu Ser Phe Asp Phe Lys Asp Thr Asp Met Lys Arg Leu
            505                 510                 515

TCC ATG GAG ATA GAG AAA GAA AAA GTG GAA TAC ATG GAA AAG AGC AAG 1818

Ser Met Glu Ile Glu Lys Glu Lys Val Glu Tyr Met Glu Lys Ser Lys
        520                 525                 530

CAT CTG CAG GAG CAG CTC AAT GAA CTC AAG ACA GAA ATC GAG GCC TTG 1866

His Leu Gln Glu Gln Leu Asn Glu Leu Lys Thr Glu Ile Glu Ala Leu
    535                 540                 545

AAA CTG AAA GAG AGG GAG ACA GCT CTG GAT ATT CTG CAC AAT GAG AAC 1914

Lys Leu Lys Glu Arg Glu Thr Ala Leu Asp Ile Leu His Asn Glu Asn
550                 555                 560                 565

TCC GAC AGG GGT GGC AGC AGC AAG CAC AAT ACC ATT AAA AAG CTC ACC 1962

Ser Asp Arg Gly Gly Ser Ser Lys His Asn Thr Ile Lys Lys Leu Thr
                570                 575                 580

TTG CAG AGC GCC AAG TCC CGA GTG GCC TTC TTT GAA GAG CTC 2004

Leu Gln Ser Ala Lys Ser Arg Val Ala Phe Phe Glu Glu Leu
            585                 590                 595
```

FIG.3E

TAGCAGGTGA CCCAGCCACC CCAGGACCTG CCACTTCTCC TGCTACCGGG ACCGCGGGAT 2064

GGACCAGATA TCAAGAGAGC CATCCATAGG GAGCTGGCTG GGGGTTTCCG TGGGAGCTCC 2124

AGAACTTTCC CCAGCTGAGT GAAGAGCCCA GCCCCTCTTA TGTGCAATTG CCTTGAACTA 2184

CGACCCTGTA GAGATTTCTC TCATGGCGTT CTAGTTCTCT GACCTGAGTC TTTGTTTTAA 2244

GAAGTATTTG TCT 2257

FIG.3F

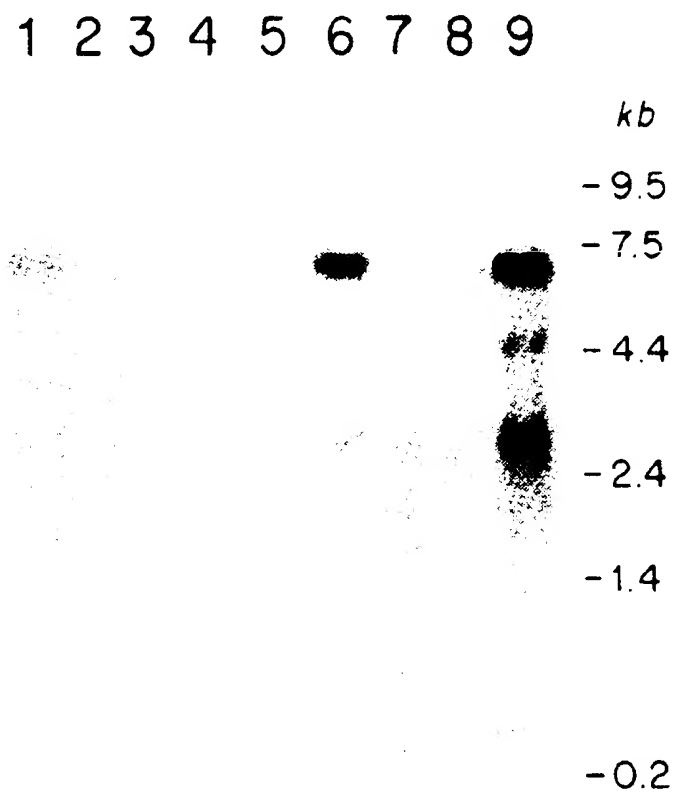


FIG. 4

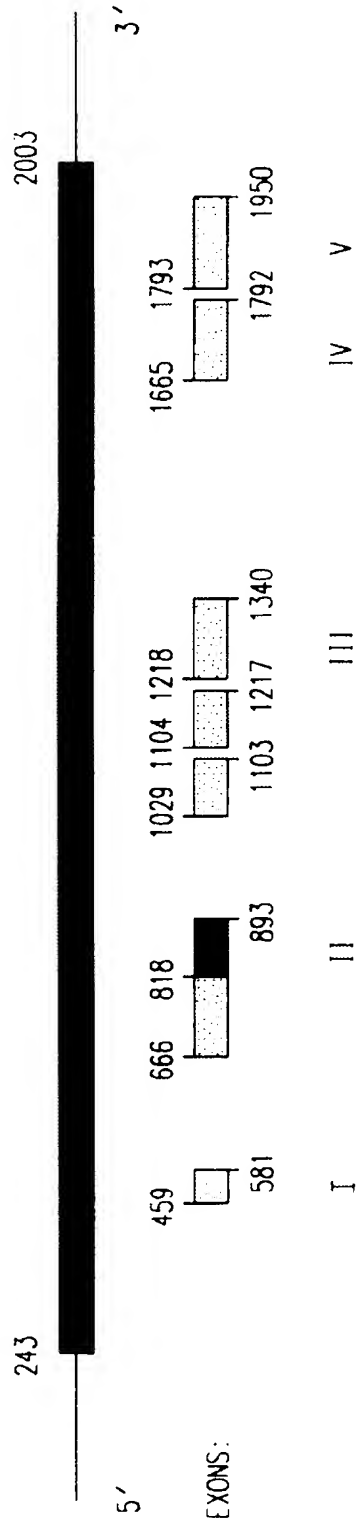

5′
3′
243
2003
EXONS:
459
581
666
818
893
1029
1104
1218
1103
1217
1340
1665
1793
1792
1950
I
II
III
IV
V

FIG.5

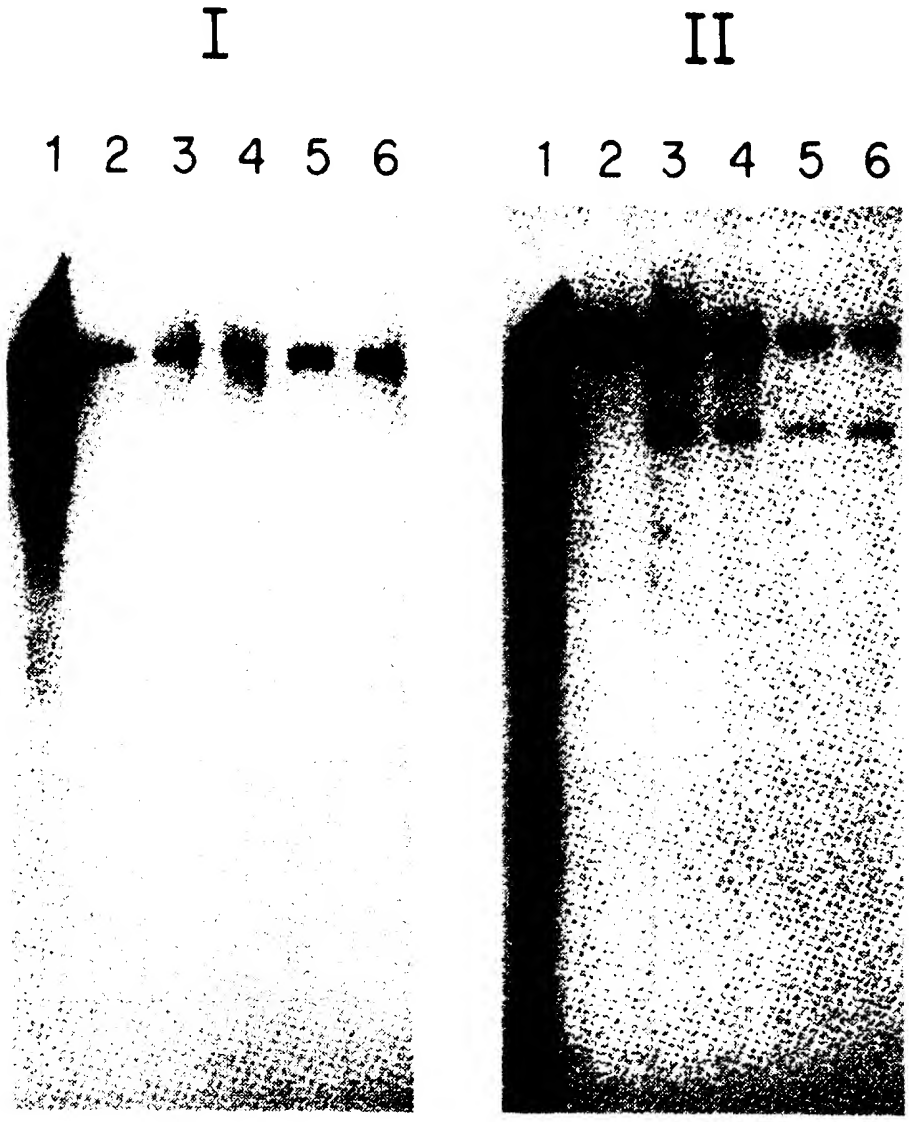


FIG. 5A

FIG. 5B

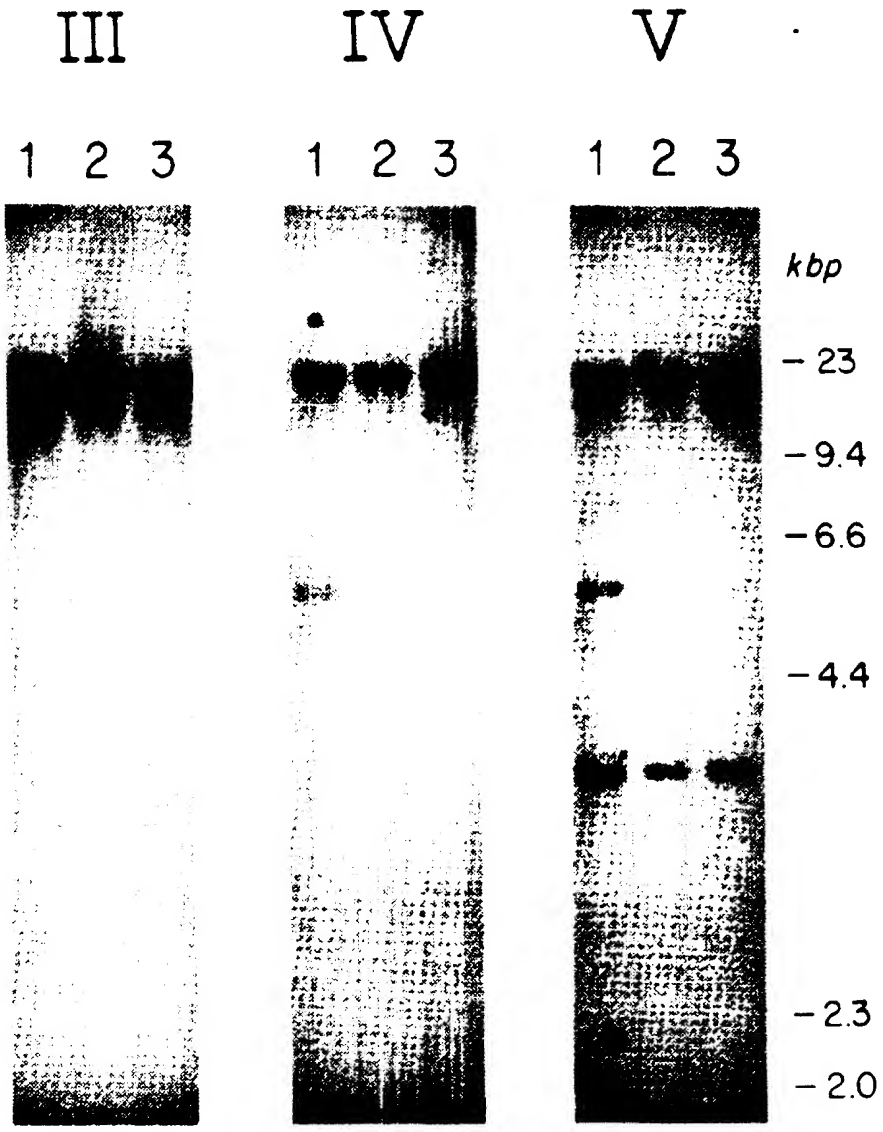


FIG. 5C FIG. 5D FIG. 5E

FIG. 6B

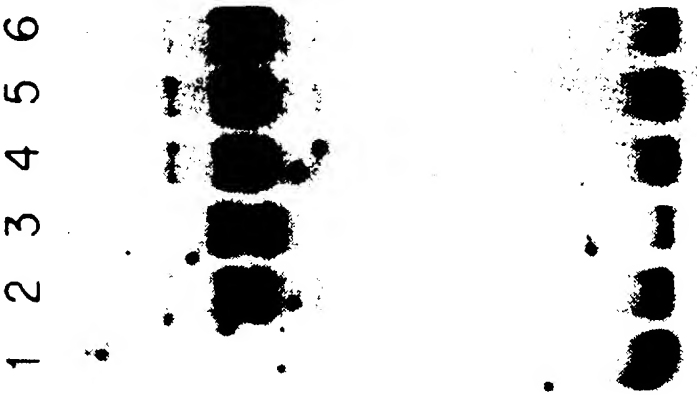


FIG. 6A

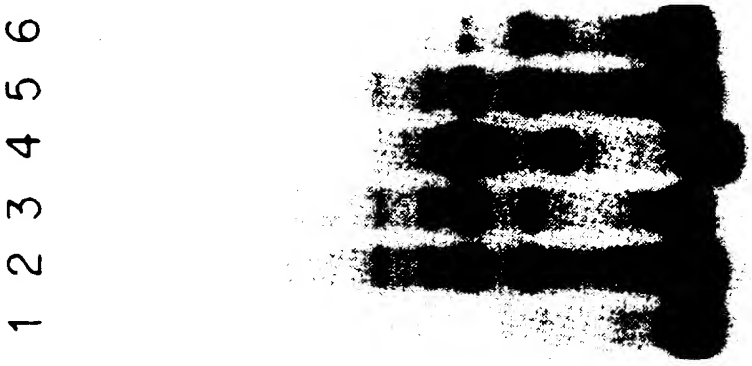
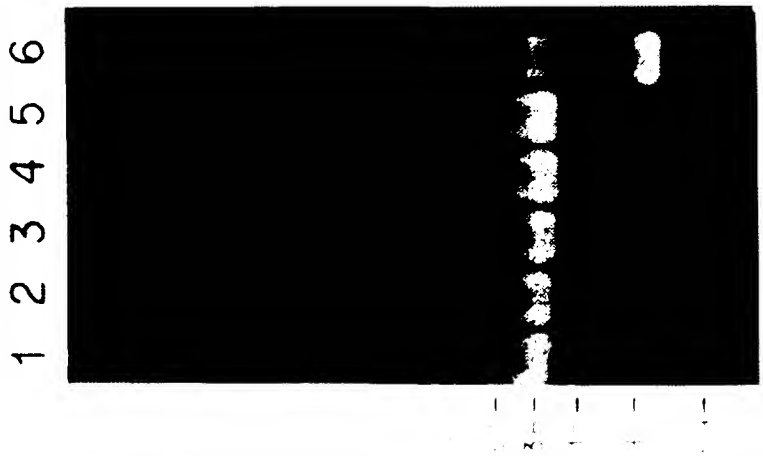


FIG. 6



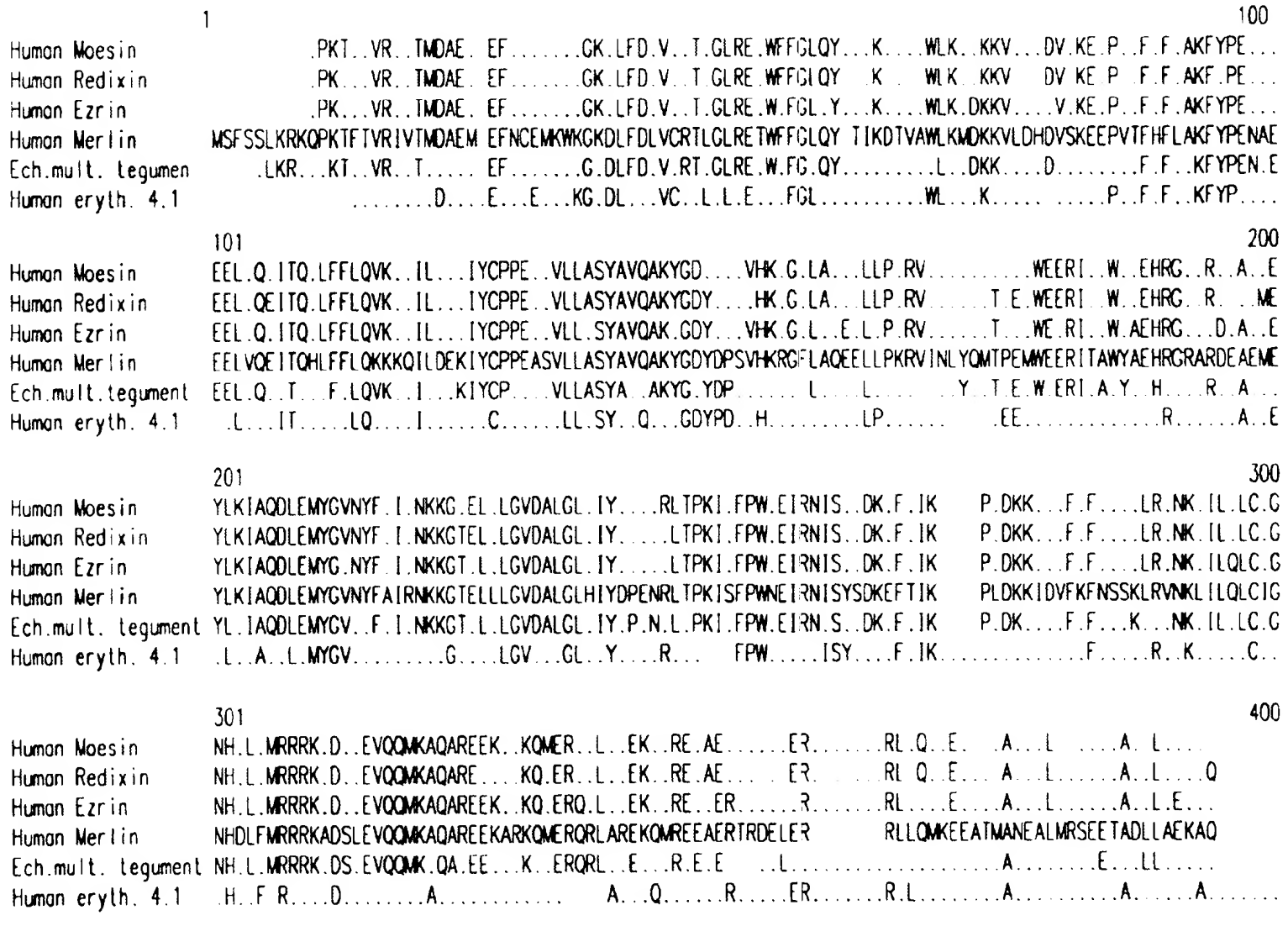

1
100
Human Moesin
Human Redixin
Human Ezrin
Human Merlin
Ech.mult. tegumen
Human eryth. 4.1
101
200
Human Moesin
Human Redixin
Human Ezrin
Human Merlin
Ech.mult.tegument
Human eryth. 4.1
201
300
Human Moesin
Human Redixin
Human Ezrin
Human Merlin
Ech.mult. tegument
Human eryth. 4.1
301
400
Human Moesin
Human Redixin
Human Ezrin
Human Merlin
Ech.mult. tegument
Human eryth. 4.1

FIG 4

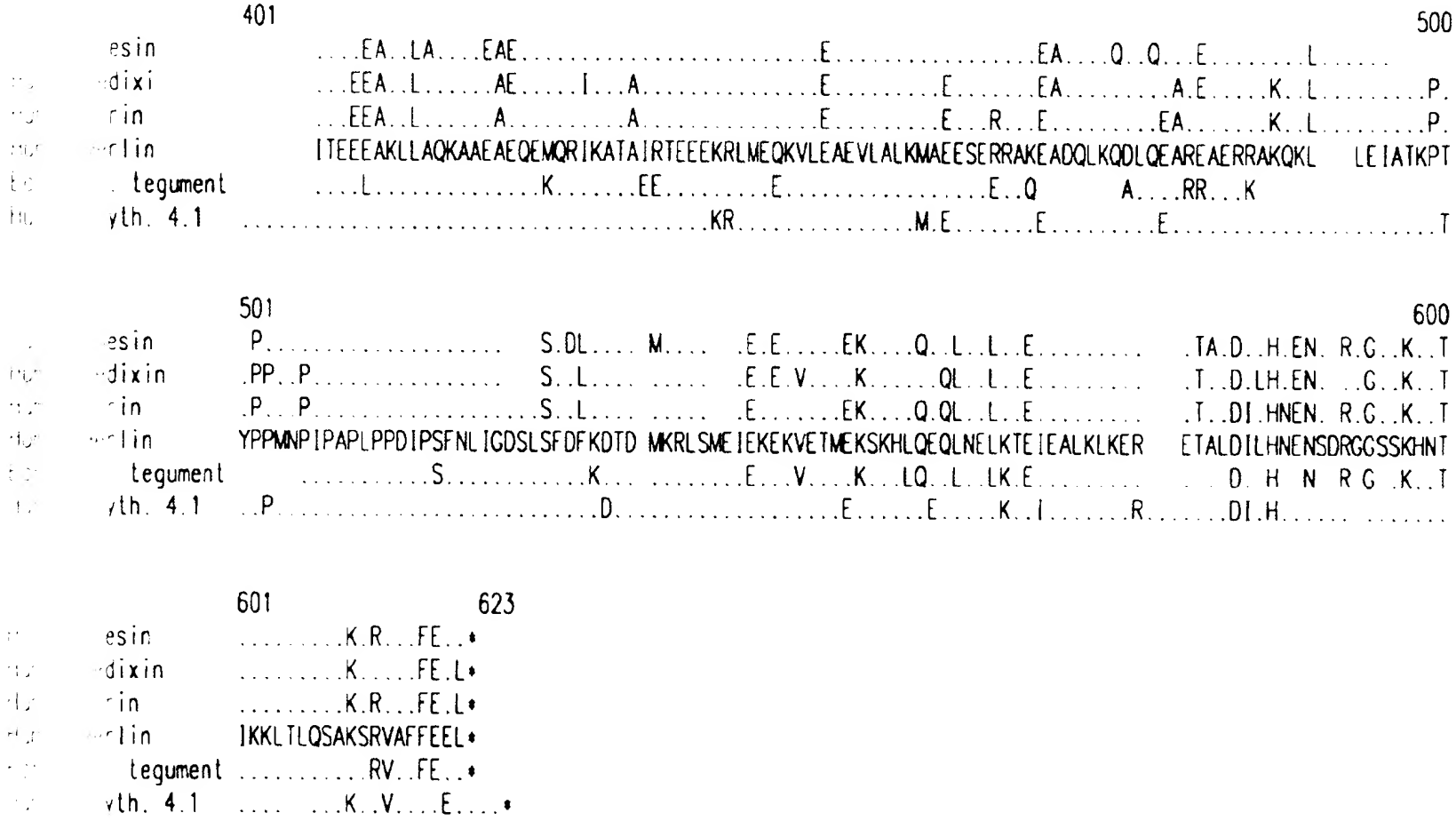


FIG.7B

EP 0 613 945 B1

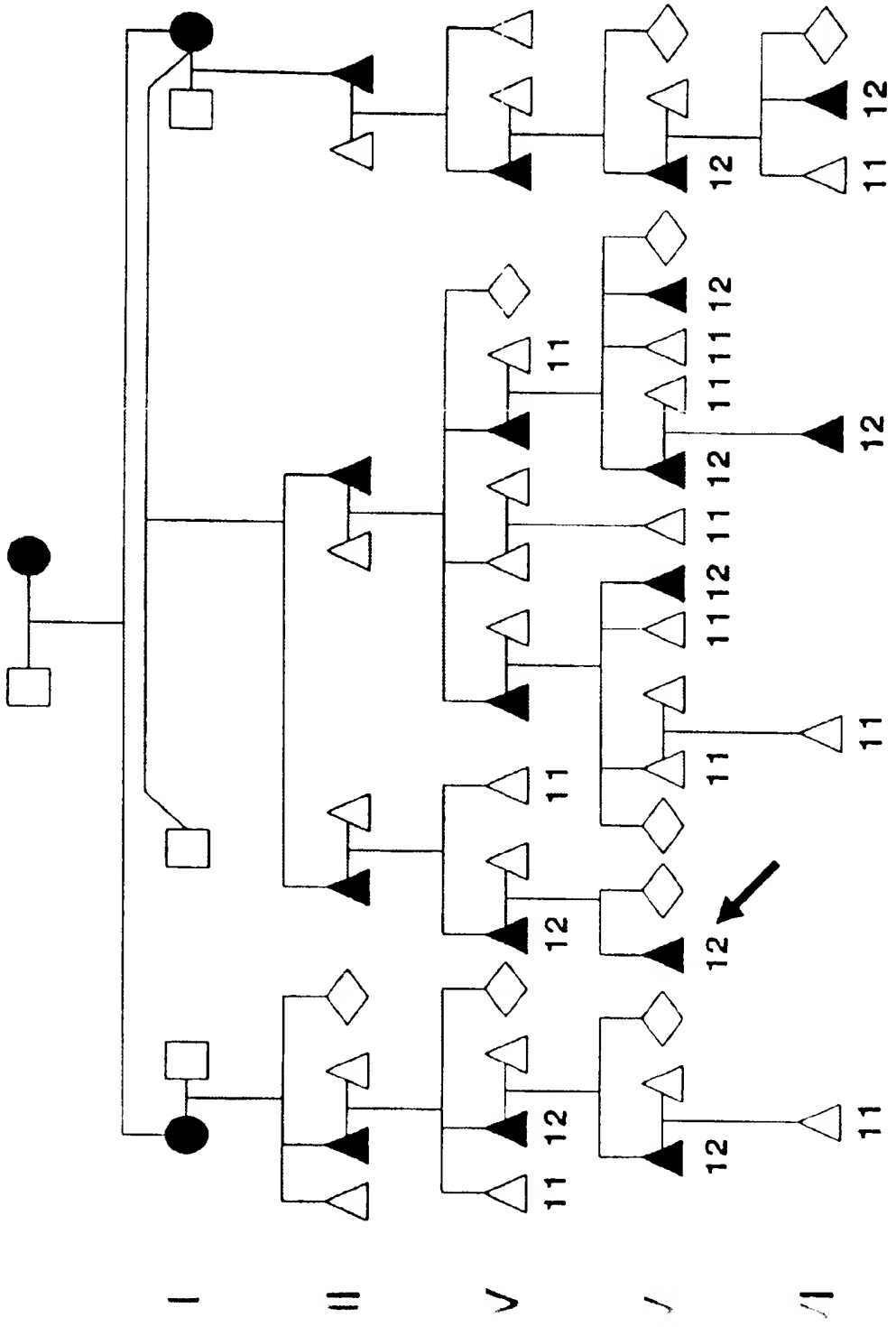


FIG.8

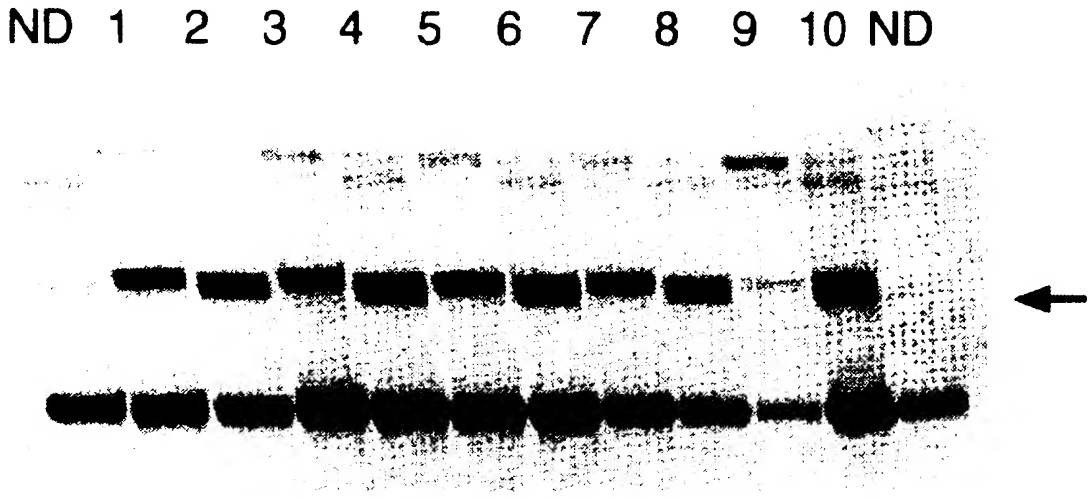


FIG. 9

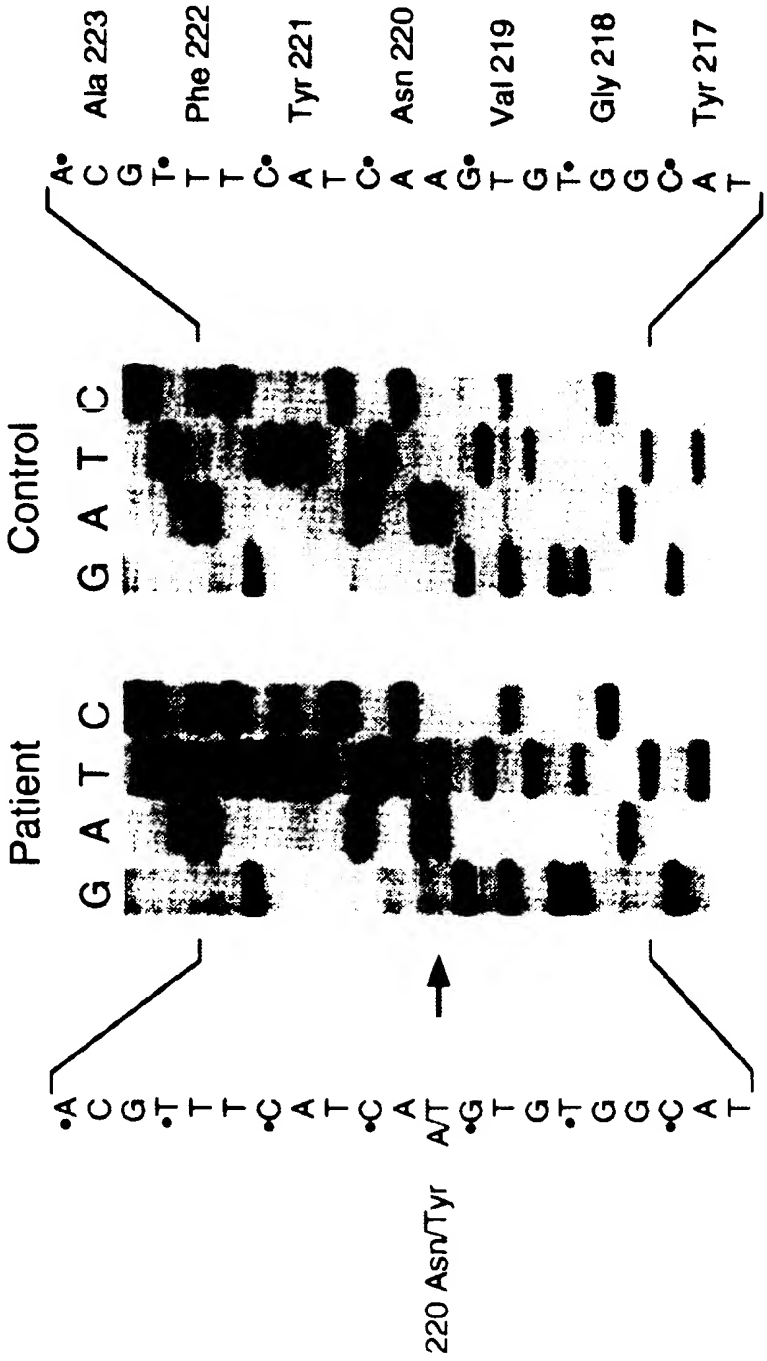


FIG. 10A

FIG. 10B

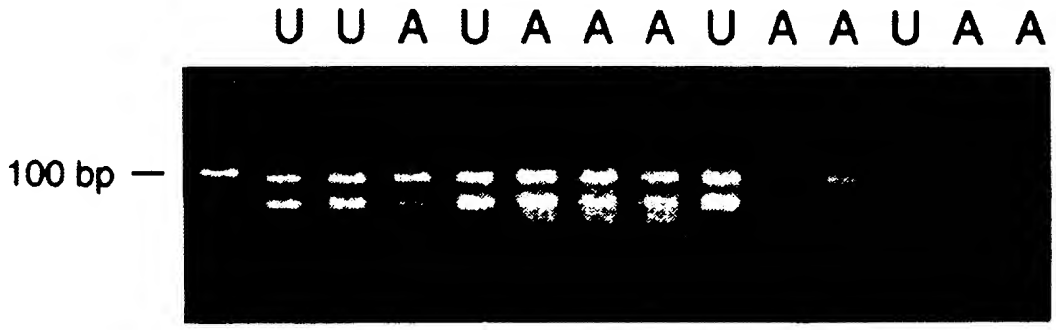


FIG. 11

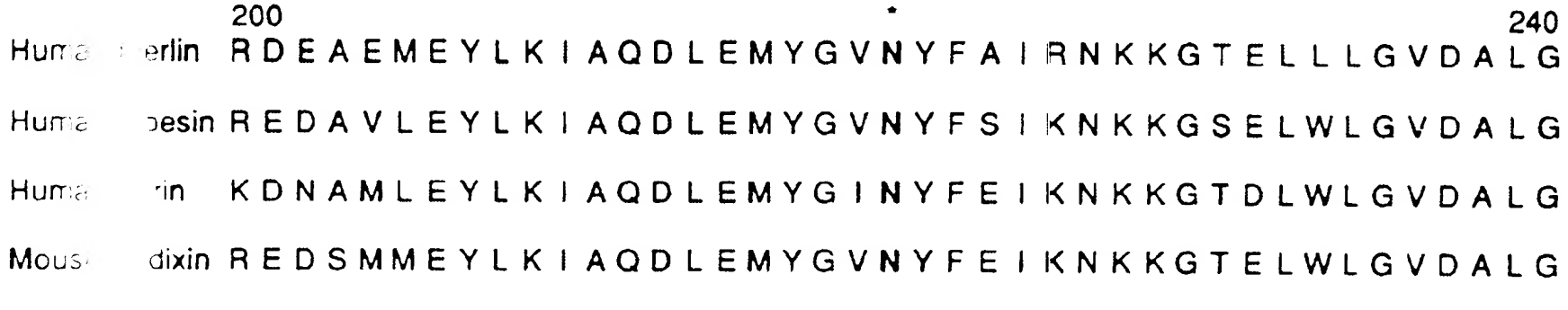


FIG. 12

EP 0 613 945 B1

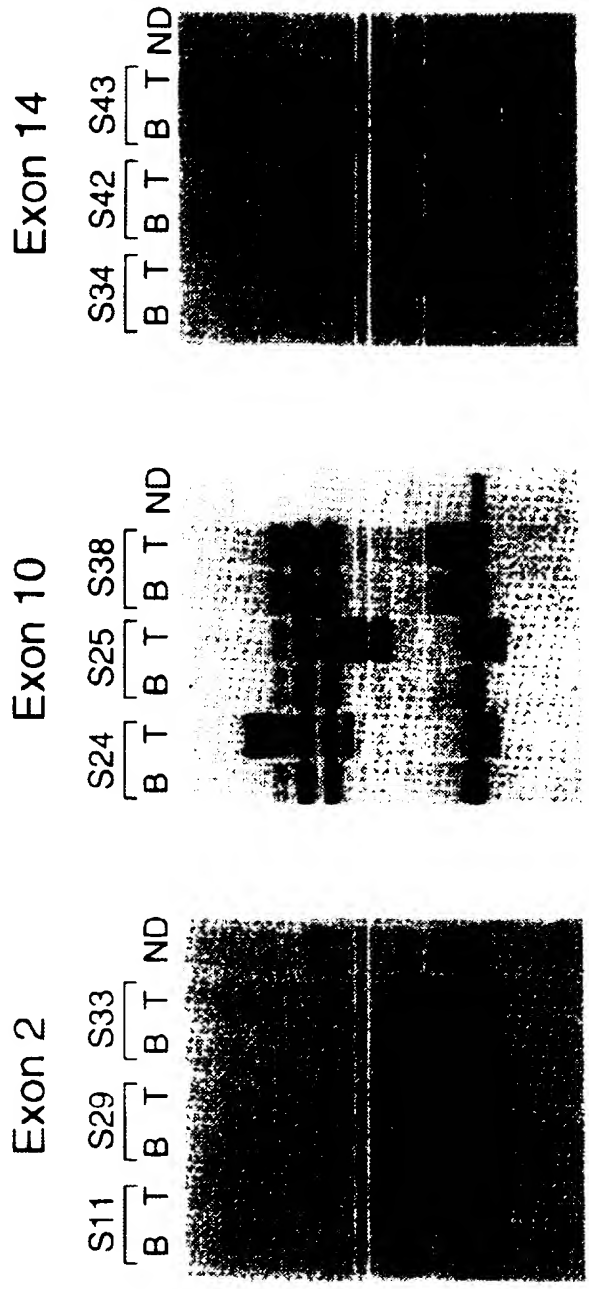


FIG. 13A

FIG. 13B

FIG. 13C

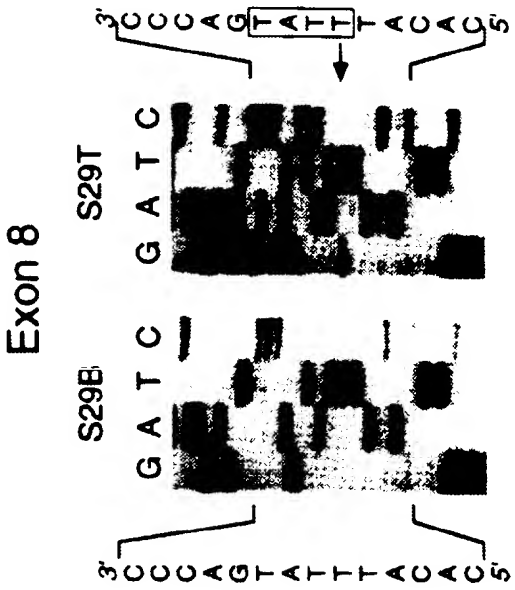


FIG.14B

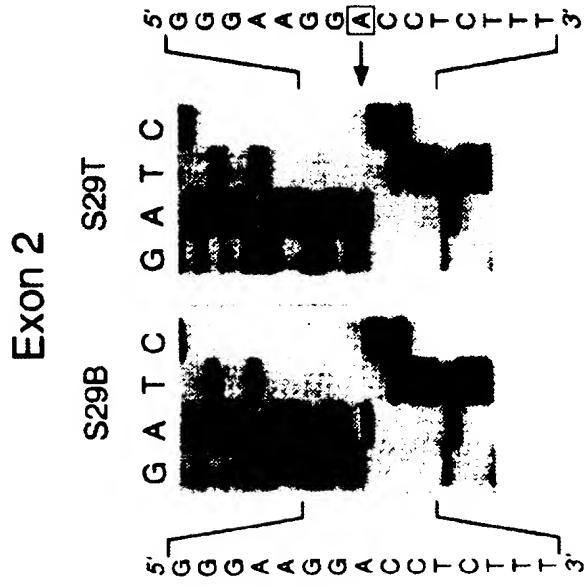


FIG.14A

AGTGCAGAGA AAAGGTTTTA TTAATGATTT TTGCTCACAG TGTCCTTCCC CATTGGTTTG 60

TTATTGCAGA TGAAGTGGAA AGGGAAGGAC CTCTTTGATT TGGTGTGCCG GACTCTGGGG 120

CTCCGAGAAA CCTGGTTCTT TGGACTGCAG TACACAATCA AGGACACAGT GGCCTGGCTC 180

AAAATGGACA AGAAGGTTGG GCTAGAACTC GATGAAACTG GTGGGGCTGA CGTGAGCTTT 240

CCA 243

FIG.15A

GCTTCTTTGA GGGTAGCACA GGAGGAAGTG CCAATATANN GTGTGTTTGT CTTTTGCTCT 60

GCAATTCTGC AGGTACTGGA TCATGATGTT TCAAAGGAAG AACCAGTCAC CTTTCACTTC 120

TTGGCCAAAT TTTATCCTGA GAATGCTGAA GAGGAGCTGG TTCAGGAGAT CACACAACAT 180

TTATTCTTCT TACAGGTACA TCAGTCAAGG CTACCCCCCA GTTCTGAGAG AGAACTTGCC 240

CAGGAGTGGT TGCAGAGTTG GCCTCAGAGT TGACC 275

FIG.15B

GCTAAAGGGC TCAGAGTGCA GGCCGTGGGG CGCGAGGGTC CCGGGCCTGA GCCCCGCGCC 60

ATGGCCGGGG CCATCGCTTC CCGCATGAGC TTCAGCTCTC TCAAGAGGAA GCAACCCAAG 120

ACGTTCACCG TGAGGATCGT CACCATGGAC GCCGAGATGG AGTTCAATTG CGAGGTAACC 180

GCCCCGCAGC CCCCAGTGGT GCCGTGACAG TCGAGGTGGA ACGTCCAGAG CTTCTC 236

FIG.15C

```
CCTCACTTCC CCTCACAGAG TATCATGTCT CCCTTGTTGC TCCTTTCAGG TAAAGAAGCA      60

GATTTTAGAT GAAAAGATCT ACTGCCCTCC TGAGGCTTCT GTGCTCCTGG CTTCTTACGC     120

CGTCCAGGCC AAGGTAGGCT CAAAGAAGAA AAATGTATTT TTNNCTGGGC GTTAATTTGC     180

GTCATGGG                                                              188
```

FIG.15D

```
TGGCAGTTAT CTTTAGAATC TCAATCGCCT GCTCTCCCTT TCTTCTTTCC AGTATGGTGA      60

CTACGACCCC AGTGTTCACA AGCGGGGATT TTTGGCCCAA GAGGAATTGC TTCCAAAAAG     120

GGTAAGAGAT TAAATTCCCT TTTCAGGAAG ACATAGCAGA TATGTGGTCT AAAAGAAAGC     180

TAACCAAAGG ACTTGAAGGA                                                 200
```

FIG.15E

```
TCTGTGTGAC TACTCCTGGT GTAGCTTTAA AATAGCTTTA CTGTTTGTAA AATGATGCAT      60

AATTATAAAA GTGGCAAACA ATACCAAATT TACTTCATGT GTAGGTTTTT TATTTTGCTC     120

TATTTTTTGG TAGGTAATAA ATCTGTATCA GATGACTCCG GAAATGTGGG AGGAGAGAAT     180

TACTGCTTGG TACGCAGAGC ACCGAGGCCG AGCCAGGTGA GGCCCATTCA TTGTTGGTTT     240

ACATTCCTTT ATGGGC                                                     256
```

FIG.15F

GAATGCTTGA TTTGGTGGCC CACCCGCTCT CCACCCATCT CACTTAGCTC CAATGACACT 60

GTCTTCCGTT CTCCCCACAG|GGATGAAGCT GAAATGGAAT ATCTGAAGAT AGCTCAGGAC 120

CTGGAGATGT ACGGTGTGAA CTACTTTGCA ATCCGGGTGT GTTGAAACCT CTCTGAGCTC 180

CTTGTGTAGT AGACAGAGAC TGAGTGAGGG CCAGCACTGC TAAAATGGTT ACTTCTTCAT 240

FIG.15G

TCTGTGGACC TGCTGAACTG CACATGTGAC AGTGTGTGCC AGATTCTTTG GAAGGTTGAA 60

TAAAATTTTG AGCCTCAGCT GGCGCTTACA GTAGCTGTTC TTATTGGATC CACAGAATAA 120

AAAGGGCACA GAGCTGCTGC TTGGAGTGGA TGCCCTGGGG CTTCACATTT ATGACCCTGA 180

GAACAGACTG ACCCCCAAGA TCTCCTTCCC GTGGAATGAA ATCCGAAACA TCTCGTACAG 240

TGACAAGGAG|GTAGGACATG TGTGTACTGC AGATGGGTCC AGCAGATCTT TCCCTGTCTG 300

CCCCCCTCAC TGGAGCCTCC CCAGCCAGGG CATCTCCTTG TTATTCATAG AGTCCTTTAA 360

TTCCCAGGCT TTGAGGGTGT GGTTGTT 387

FIG.15H

GACTTGGTGC TCCTAATTCC CTGAGGTTTA GTGCCTGGAT ACTGGGAAGC CAGNACAAGG 60

GCATAACNTC ATGCTGGTCT GTGGCCAGTG TGGTTGCGCA TTTGTGGAAT TNCCAATTGC 120

TGGTAACATT CCAGGCTGTC GGACTGAAAC TGTGTTCTGC TTCATTCTTC CAGTTTACTA 180

TTAAACCACT GGATAAGAAA ATTGATGTCT TCAAGTTTAA CTCCTCAAAG CTTCGTGTTA 240

FIG.15I

```
TGCTACCTGC AAGAGCTCAA ACTGCTATGG CACTAGTGGG CCAGTAGGCA GTGAAGTAAA      60
TTTGTGGATA TTAACCTTTT TGTCTGCTTC TGTGGCCACA GATTCTCCAG CTATGTATCG     120
GGAACCATGA TCTATTTATG AGGAGAAGGA AAGCCGATTC TTTGGAAGTT CAGCAGATGA     180
AAGCCCAGGC CAGGGAGGAG AAGGCTAGAA AGCAGGTGAG CACAACCTTG TTTTAACTGA     240
TGATGTCACT GTGTGGTCAG                                                 260
```

FIG.15J

```
TCTTTGGCCC TTGTGGCACC CTAGGTCTCG AGCCCTGTGA TTCAATGACT GTTTTTCTTC      60
ACCCCTCGCA GATGGAGCGG CAGCGCCTCG CTCGAGAGAA GCAGATGAGG CAGGAGGCTG     120
AACGCACGAG GGATGAGTTG GAGAGGAGGC TGCTGCAGAT GAAAGAAGAA GCAACAATGG     180
CCAACGAAGC ACTGGTGATT TCTGAGGGGC TGGGGTTCCA GGAGGCTACT TGGGGACTTC     240
CTTGGCTTTT CTGGAGCTTG GTCTCCTGAA AACATGAGTT AGCAGCGTTT GC              292
```

FIG.15K

```
CGGGAGAACA GCACATGATC CCACTTCAGC TAAGAGCACT GTGCCCTCCA GATGCGGTCT      60
GACGAGACAG CTGACCTGTT GGCTGAAAAG GCCCAGATCA CCGAGGAGGA GGCAAAACTT     120
CTGGCCCAGA AGGCCGCAGA GGCTGAGCAG GAAATGCAGC GCATCAAGGC CACAGCGATT     180
CGCACGGAGG AGGAGAAGCG CCTGATGGAG CAGAAGGTGC TGGAAGCCGAG GTGCTGGCA     240
CTGAAGATGG CTGAGGAGTC AGAGAGGAGG TGAGGGGGCA CCGGGCACCA GACTGGCGAG     300
GAGGCTGGCC AAGGGCCGCA GACCAGCCTG CCCTGAGGCT GAGCTCTACA GCAGTTGTCC     360
```

FIG.15L

GGTGTCTTTT CCTGCTACCT GCCCTCTTCT GTGAAGCTGA CATCTCATCC TTTCCTTGCA 60

GGGCCAAAGA GGCAGATCAG CTGAAGCAGG ACCTGCAGGA AGCACGCGAG GCGGAGCGAA 120

GAGCCAAGCA GAAGCTCCTG GAGATTGCCA CCAAGCCCAC GTACCCGGTG AGCCTGGGGG 180

CCACCAGCTG GGGCTGCCTT AGTCCTGGTG ATGTTCTCTT TCCTCCC 227

FIG.15M

TGTGCCATTG CCTCTGTGGC TGCTGGAGGA ICGGTTGTCA ACACAGTAGT GTCCTTCTGT 60

GCTTGTATGA CCCAAGCTCC TAATCCGAAA TTTCTCATTA ACAGCCCATG AACCCAATTC 120

CAGCACCGTT GCCTCCTGAC ATACCAAGCT TCAACCTCAT TGGTGACAGC CTGTCTTTCG 180

ACTTCAAAGA TACTGACATG AAGCGGCTTT CCATGGAGAT AGAGAAAGAA AAGTATGTAG 240

CCCCCTGTGC CCTGCTGTGG GCTTGCTGTG AACTAGACTG A 281

FIG.15N

TGGCCAAGTA GAGACGTGAN NCCAGCNTNA AACCCTAGAT CGCACACCAA GCAGCTTGTG 60

GGCCACAGAG CACCTGAGCC GTGTCTCACT GTCTGCCCAA GCCCTGATGC ATGATACCCT 120

CTTGCCCGCA GAGTGGAATA CATGGAAAAG AGCAAGCATC TGCAGGAGCA GCTCAATGAA 180

CTCAAGACAG AAATCGAGGC CTTGAAACTG AAAGAGAGGG AGACAGCTCT GGATATTCTG 240

CACAATGAGA ACTCCGACAG GGGTGGCAGC AGCAAGCACA ATACCATTAA AAAGGTACCC 300

FIG.15O

CAAACAAAAT CACTCATCAC GATNTCAGGC CTATCCAAGC ATTTTGCANA TGGCACTTAT 60

GGCATTGTTG ATATCACAGG GTATGTTTTT GTTTTTCTTC ATTTTATTTT GCTGGTTTAG 120

CCTCAAGCCC AAGGCAGAAG ACCTATCTGC ATTTGAGCCC TCAAAGTAGC TTGTTCCCAG 180

GTACTCTCTA TGTGGTGATG GTGCTGCCCT CTGTGATACT AACCCGTGCA TGAGNTTGCC 240

TGTCTCTGTC TCGG 254

FIG.15P

AGGACCCTGT GAGACAGAGC GGAGGTCNNG TGCCCTCTCA GCTTCTTCTC TGCTTTCTTA 60

CAGCTCACCT TGCAGAGCGC CAAGTCCCGA GTGGCCTTCT TTGAAGAGCT CTAGCAGGTG 120

ACCCAGCCAC CCCAGGACCT GCCACTTCTC CTGCTACCGG GACCGCGGGA TGGACCAGAT 180

ATCAAGAGAG CCATCCATAG GGAGCTGGCT GGGGGTTTCC GTGGGAGCTC CAGAACTTTC 240

CCCAGCTGAG TGAAGAGCCC AGCCCCTCTT ATGTGCAATT GCCTTGAACT ACGACCCTGT 300

AGAGATTTCT CTCATGGCGT TCTAGTTCTC TGACCTGAG 339

FIG.15Q